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A review of the fusion of trigeminal and facial ganglia during larval development of some neobatrachian anurans

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The intracranial relations of the trigeminal (V) and facial (VII) nerves have been examined in larval sequences of *Ceratophrys cranwelli*, *Dermatonotus muelleri*, *Hyla pulchella andina*, *Lepidobatrachus llanensis*, *Phyllomedusa sauvagii*, *Physalaemus biligonigerus* and *Scinax fuscovaria*. Whole mounts stained for peripheral nerves and transverse histologic sections were prepared for this purpose. *H. pulchella andina*, *P. sauvagii*, *S. fuscovaria* and *P. biligonigerus* have the trigeminal and facial ganglia fused at similar stages of larval development. In later larval stages this fusion progresses to the roots of these nerves. In early stages of development of *D. muelleri* the trigeminal and facial roots are fused; in later larval stages this fusion occurs at the ganglia. *C. cranwelli* and *L. llanensis* have the trigeminal and facial nerves separated throughout their larval development. In *C. cranwelli* fusion of the ganglia takes place before metamorphosis, whereas the separation continues in *L. llanensis* until metamorphosis ends.



INTRODUCTION

The importance of larval characters in anuran classification has been stressed by ORTON (1957), STARRETT (1973) and SOKOL (1975). Some of these characters have been incorporated into phylogenetic analyses of the taxon in order to define monophyletic groups (DUELLMAN & TRUEB, 1985; FORD & CANNATELLA, 1993). Among such characters one refers to the relation between the trigeminal (V) and facial (VII) nerves.

In the majority of anurans, as distinct from other vertebrates, the ganglia of the trigeminal and facial nerves are fused in a single prootic ganglion, and all rami of both



nerves emerge from the skull through a single prootic foramen, which lies immediately anterior to the otic capsule (Goodrich, 1930; De Beer, 1937). The fusion of these ganglia was proposed by Sokol (1975) as a derived condition for a group of anurans and their larvae. Anurans with Type III larvae (Discoglossoidei) have separated trigeminal and facial nerves, with their rami transversing the skull through two foramina separated by the prefacial commissure. Anurans with Type IV, II and I larvae (Ranoidei) have a prootic ganglion and a single cranial foramen for exit of the trigeminal and facial rami. Different degrees of fusion of the nerve roots among the Ranoidei tadpoles were mentioned by Sokol (1975, 1977, 1981).

The Ranoidei proposed by Sokol (1975) were later renamed Pipanura by Ford & Cannatella (1993). Fusion of trigeminal and facial ganglia is included among their synapomorphies, although the information related to this character among the taxa involved is scant.

The existing information on trigeminal and facial nerves in anurans is summarized in tab. 1. This calls attention to: (1) the limited number of species in which the character has been investigated; (2) the lack of uniformity in the information, which makes comparison difficult, because different stages of ontogeny were studied.

Because data in the literature exist for only six genera, we decided to review this character in neobatrachian anurans. For this purpose, we investigated premetamorphic larval sequences of species belonging to three major anuran groups which are not closely related.

Ceratophrys cranwelli, *Lepidobatrachus llanensis* and *Physalaemus biligonigerus* are included in the "Leptodactylidae", considered a paraphyletic group (Ford & Cannatella, 1993). *C. cranwelli* and *L. llanensis* are in a subfamily different from that of *P. biligonigerus* (Duellman & Trueb, 1985).

Hyla pulchella andina, *Phyllomedusa sauvagii* and *Scinax fuscovaria* are members of the Hylidae, a taxon defined by a single synapomorphy (Ford & Cannatella, 1993). Both *H. p. andina* and *S. fuscovaria* are grouped in a subfamily different from that of *P. sauvagii* (Duellman & Trueb, 1985).

Dermatonotus muelleri is included in the Microhylidae, whose monophyly has been supported by some synapomorphies (Ford & Cannatella, 1993).

We analyzed trigeminal and facial nerves relation during development in these species and reviewed the available literature on this subject in order to evaluate an important character that has been considered in recent anuran phylogenies.

Material and methods

The larval specimens were examined at different stages up to the beginning of metamorphosis, in accordance with Gosner's (1960) table. The larval stages analyzed are specified for each species because representative specimens of the complete sequence were not available. The skulls of larvae and adult specimens were also analyzed in order to

Tab. 1. - Condensed information from literature in which there are observations of trigeminal and facial ganglia and roots, and prefacial commissure in anurans.

Family (LAURENT, 1986)	Genus	Author	Specimens analyzed	V and VII ganglia	V and VII roots	Prefacial commissure
Leiopelmatidae	*Ascaphus*	PUSEY, 1938	Ontogeny	Separated	Separated	Present
		VAN EEDEN, 1951	Ontogeny	Separated	Separated	Present
	Leiopelma	PUSEY, 1938	Ontogeny	Separated	Separated	Present
		VAN EEDEN, 1951	Ontogeny	Separated	Separated	Present
		STEPHENSON, 1951	Ontogeny	Separated	Separated	Present
Discoglossidae	*Bombina*	PUSEY, 1938	Adult	Separated	Separated	Present
		VAN EEDEN, 1951	Adult	Separated	Separated	Present
		SOKOL, 1975	Larval stage	Separated	Separated	Present
	Alytes	DE BEER, 1937	Larval stage	Separated	Separated	Present
		PUSEY, 1938	Adult	Contiguous	Separated	Present
		VAN EEDEN, 1951	Adult	Contiguous	Separated	Present
	Discoglossus	PUSEY, 1943	Adult	Fused	Separated	Present
		VAN EEDEN, 1951	Adult	Fused	Separated	Present
		SCHLOSSER & ROTH, 1995	Larval stage	Separated	Separated	?
Pelobatidae	*Pelobates*	PLASOTA, 1974	Ontogeny	Fused	?	Absent
		ROČEK, 1980	Ontogeny	Fused	?	Absent
		SOKOL, 1975	Larval stage	Fused	Separated	Absent
	Scaphiopus	SOKOL, 1975	Larval stage	Fused	Fused	Absent
Pelodytidae	*Pelodytes*	SOKOL, 1981	Larval stage	Fused	?	Absent
Rhinophrynidae	*Rhinophrynus*	SOKOL, 1975	Larval stage	Fused	?	Absent
Pipidae	*Xenopus*	SOKOL, 1977	Larval stage	Fused	?	Absent
	Pipa	SOKOL, 1977	Larval stage	Fused	?	Absent
	Hymenochirus	SOKOL, 1977	Larval stage	Fused	?	Absent
		PATERSON, 1951	Adult	Fused	Separated	Present
Myobatrachidae	*Heleophryne*	VAN DER WESTHIZEN, 1961	Ontogeny	Fused	?	Absent
	Pseudophryne	JACOBSON, 1968	Larval stage	Fused	?	Absent
	Pleurodema	SOKOL, 1975	Larval stage	Fused	Separated	Absent
Ranidae	*Rana*	DE BEER, 1937	Ontogeny	Fused	?	Absent
		PUSEY, 1938	Ontogeny	Fused	?	Absent
		DE JONGH, 1968	Ontogeny	Fused	?	Absent
		PLASOTA, 1974	Ontogeny	Fused	?	Absent
Microhylidae	*Breviceps*	SWANEPOEL, 1971	Ontogeny	Fused	Fused	Absent
	Hypopachus	SOKOL, 1975	Larval stage	Fused	Fused	Absent

verify the state of the foramen for exit of trigeminal and facial nerves. The preparations studied are listed in app. 1. They have been deposited in the herpetological collections of the Museo de Ciencias Naturales (MCN), Universidad Nacional de Salta, Argentina, and of the Fundación Miguel Lillo (FML), Argentina.

The whole mounts stained for peripheral nerves were prepared with Sudan Black B and maceration in trypsin (Filipski & Wilson, 1984, 1985; Nishikawa, 1987). The 10 µm transverse serial sections were obtained using current histologic techniques, with hematoxylin-eosine coloration. The whole mounts stained for bone and cartilage were processed by the technique described in Wassersug (1976).

Observations and photographs were made using a stereomicroscope and an optical microscope.

We consider it necessary to explain that the term "root" is used for describing the preganglionic part of the nerve.

Results

"Leptodactylidae"

Ceratophrys cranwelli

The specimens at larval stage 31-34 show the trigeminal and facial nerves completely separated (fig. 1a-b) and the trigeminal ganglion is clearly defined. The skeletal preparations of larvae and adults present an undivided prootic foramen.

The facial nerve (VII) enters the rhombencephalon with the vestibulo-cochlear nerve (VIII) posterior to the root of the trigeminal nerve (fig. 2a). The facial root and the lateral-line nerves are together. They take a rostro-ventral course and diverge at the prootic foramen level in the prominent truncus hyomandibularis and the antero-dorsal lateral-line nerve. The truncus hyomandibularis lies below the ascendent process and subocular arch of palatoquadrate and extends toward the anterior elements of the hyobranchial skeleton (fig. 2b). The antero-dorsal lateral-line nerve is thin and branches in two rami.

The trigeminal nerve has its root in an anterior position and dorsal to the facial root (fig. 1a-b, 2b). The ganglion of this nerve is prominent (fig. 1b, 2b), and two branches diverge from it, namely: (1) the ramus ophthalmicus profundus lies ventral to the ascendent process of the palatoquadrate and runs anteriorly along the orbit wall toward the ethmoidal area (fig. 1a); (2) the truncus maxillo-mandibularis lies above the ascendent process of the palatoquadrate and diverges in the middle of the orbit into maxillaris and mandibularis branches (fig. 1a).

The specimen at stage 42 presents complete fusion of the trigeminal, facial, lateral-line, and vestibulo-cochlear roots. The roots and ganglia of trigeminal and facial nerves show no separation, and it is possible to differentiate the components of each nerve only at the prootic foramen level.

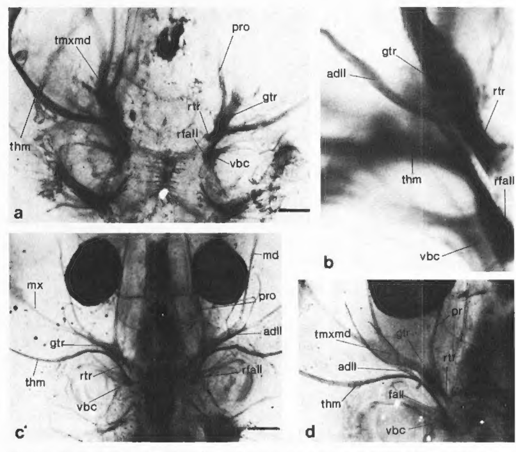


Fig. 1. — (a) Whole mount larval specimen of *Ceratophrys cranwelli* (stage 33) stained for peripheral nerves. The trigeminal, facial, lateral-line, and vestibulo-cochlear nerves are shown. Bar: 0.59 mm. — (b) Detail of trigeminal and facial nerves of the same specimen as seen from dorsal view. The trigeminal ganglion shows no connections with facial nerve. — (c) Whole mount larval specimen of *Lepidobatrachus llanensis* (stage 33) stained for peripheral nerves. Ventral view in which the trigeminal, facial, lateral-line, and vestibulo-cochlear nerves are observed. Bar: 1.20 mm. — (d) Detail of the trigeminal and facial nerves in same specimen as in (c). No fusion of trigeminal and facial nerves can be observed. — Abbreviations: adll, antero-dorsal lateral-line nerve; gtr, trigeminus ganglion; md, ramus mandibularis of trigeminus; mx, ramus maxillaris of trigeminus; pro, ramus ophthalmicus profundus of trigeminus; rfall, facial and lateral-line roots; rtr, trigeminal root; thm, truncus hyomandibularis; tmxmd, truncus maxillo-mandibularis of trigeminus; vbc, vestibulo-cochlear nerve.

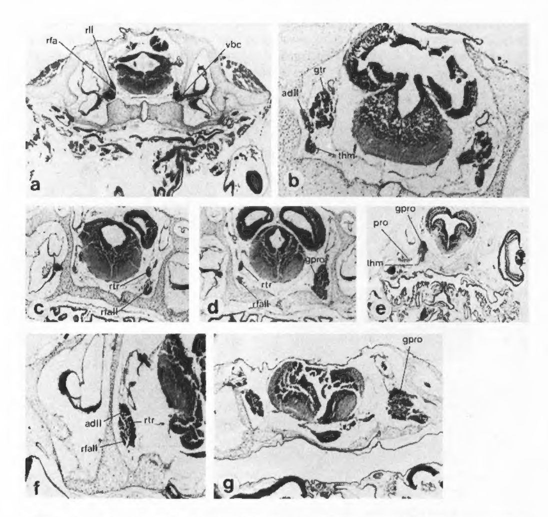


Fig. 2. – (a) Transverse section of larval specimen of *Ceratophrys cranwelli* (stage 33) at the level of the anterior half of the otic capsule; the facial and lateral-line roots are dorsal to the vestibulo-cochlear nerve. – (b) Transverse section of larval specimen of *Ceratophrys cranwelli* (stage 33) at the level of prootic foramen; trigeminal ganglion is defined and separated from the truncus hyomandibularis and antero-dorsal lateral line nerve. – (c) Transverse section of larval specimen of *Phyllomedusa sauvagii* (stage 33) at the level of anterior half of the otic capsules, in which the position of trigeminal, facial and lateral line roots is marked. – (d) Transverse section in larval specimen of *Phyllomedusa sauvagii* (stage 33) at the level of a plane anterior to that of (c). Structure of the prootic ganglion is shown. – (e) Transverse section of larval specimen of *Phyllomedusa sauvagii* (stage 33) at the level of the prootic foramen, in which the outlet of the ramus ophthalmicus profundus and truncus hyomandibularis of the prootic ganglion are observed. – (f) Tranverse section of larval specimen of *Dermatonotus muelleri* (stage 33) at the level of the otic capsule, in which the fusion of the trigeminal, lateral-line and facial roots is observed. – (g) Transverse section of larval specimen of *Dermatonotus muelleri* (stage 33) at the level of the anterior limit of the otic capsule, in which the prootic ganglion is observed. – Abbreviations: gpro, prootic ganglion; rfa, facial root; rll, lateral-line root; other abbreviations as in fig. 1.

Lepidobatrachus llanensis

The specimens at larval stages 31, 33 and 35 present complete separation of trigeminal and facial nerves (fig. 1c-d). The skeletal preparations of larvae and adults present a single prootic foramen.

The facial and lateral-line roots are in a posterior and ventral location with respect to the trigeminal nerve root (fig. 1d). The same trigeminal, lateral-line, and facial rami as those described for *C. cranwelli* are observed here (fig. 1c).

In the stage 37 specimen an approximation of the trigeminal and facial roots is observed. In the stage 42 specimen the ganglion of the trigeminal nerve and its root maintain their distinctness. The prootic ganglion is completely formed in the adult specimen.

Physalaemus biligonigerus

The specimens analyzed present trigeminal and facial roots separated from each other (fig. 3a), but anteriorly the prootic ganglion is already evident at the earliest stages (31-35). In whole mounts stained for bone and cartilage the prootic foramen is present. All the trigeminal, facial, and lateral-line rami described for *C. cranwelli* are recognized (fig. 3a). The separation of the nerve roots becomes less evident from stage 37 onward.

HYLIDAE

Hyla pulchella andina, Phyllomedusa sauvagii and *Scinax fuscovaria*

The ontogenetic sequences analyzed in these species present similar characteristics. The prefacial commissure is absent in larval and adult skeletal preparations. The formation of a prootic ganglion is evident (fig. 2d-e, 3b), although trigeminal and facial roots are separated (fig. 2c-d, 3b). In more advanced stages of development, the proximal portion of each nerve can only be recognized at the level of the roots.

MICROHYLIDAE

Dermatonotus muelleri

The prootic foramen is evident in osteologic preparations of larval and adult specimens. In the stage 27 specimen the truncus hyomandibularis and the trigeminal rami are clearly differentiated. They are independent; they enter the encephalon very close together. The lateral-line nerve crosses its fibers from the facial nerve to trigeminal nerve. No structure recognizable as a prootic ganglion can be distinguished. In stage 29, facial and trigeminal roots are fused. In the stage 33 specimen, facial and trigeminal nerves are integrated to form the prootic ganglion (fig. 2f-g, 3c-d).

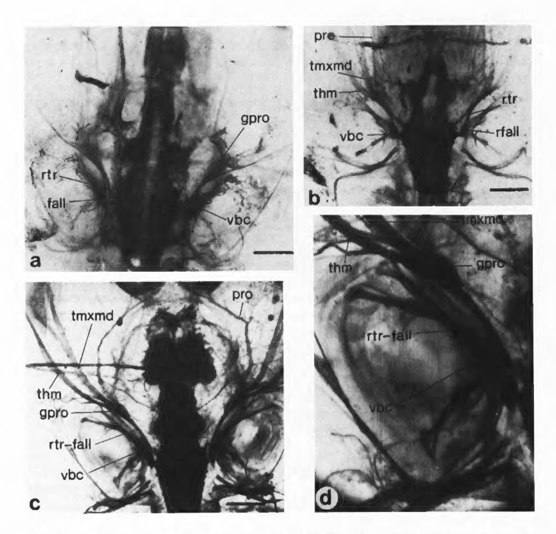


Fig. 3. — (a) Whole mount of larval specimen of *Physalaemus biligonigerus* (stage 33) stained for peripheral nerves. The trigeminal, facial and vestibulo-cochlear nerves are indicated; the trigeminal and facial-lateral-line roots are separated, but the presence of the prootic ganglion is evident. Bar: 0.48 mm. — (b) Whole mount of larval specimen of *Phyllomedusa sauvagii* (stage 33) obtained for peripheral nerves. The relations of trigeminal and facial nerves are as in (a). Bar: 1 mm. — (c) Whole mount of larval specimen of *Dermatonotus muelleri* (stage 33) obtained for peripheral nerves. The trigeminal, facial-lateral-line, and vestibulo-cochlear nerves are illustrated. The trigeminal and facial-lateral-line roots are fused and the presence of the prootic ganglion is evident. Bar: 0.52 mm. — (d) Detail of the trigeminal and facial relationships in the same specimen as in (c). — Abbreviations: rtr-fall, trigeminal, facial and lateral-line roots; other abbreviations as in fig. 1-2.

DISCUSSION AND CONCLUSIONS

The fusion of the trigeminal and facial ganglia presented by the anurans is an important character in their phylogeny, although, as mentioned, it has not been sufficiently investigated.

Some data from the literature are not entirely in agreement with observations made by SOKOL (1975). For instance, fusion of the trigeminal and facial ganglia has been described in postmetamorphic stages of *Discoglossus* (PUSEY, 1943; VAN EEDEN, 1951) – although trigeminal and facial ganglia can be clearly distinguished in *D. pictus* tadpoles (SCHLOSSER & ROTH, 1995) – and the presence of the prefacial commissure has been observed in *Hymenochirus* adults (PATERSON, 1951).

Information available for Pipanura is limited to seven genera of Mesobatrachia and only six genera of Neobatrachia. This information is insufficient for a discussion in depth, because for some taxa the character is described in a single larval stage, whereas for others the condition for adult forms or final stages of larval development has been superficially mentioned (see tab. 1).

The presence of a prootic ganglion and separate trigeminal and facial roots observed in *Physalaemus biligonigerus*, *Hyla pulchella andina*, *Phyllomedusa sauvagii* and *Scinax fuscovaria* larvae is in agreement with the observations in *Pleurodema* (SOKOL, 1975) and *Rana* (PUSEY, 1938). These species have the roots fused only near the ganglion. In later ontogenetic stages, this fusion extends proximally.

The trigeminal and facial nerves are found completely separated in larvae of *Ceratophrys cranwelli* and *Lepidobatrachus llanensis*, a characteristic which continues in *L. llanensis* until the start of metamorphosis. As this condition has not been referred to in Pipanura (tab. 1), the observations made on these species give grounds for not accepting the generalization proposed by SOKOL (1975) that Type IV, II and I larvae have a single prootic foramen and fused facial and trigeminal ganglia.

The early fusion of the trigeminal and facial roots and subsequent formation of the prootic ganglion observed in *Dermatonotus muelleri* larval development are in agreement with the information available on *Breviceps adspersus* (SWANEPOEL, 1971). Although ontogenetic aspects of the trigeminal and facial relations have not been described for *B. adspersus*, it is possible to deduce the process of fusion from roots to ganglia from the schema included (SWANEPOEL, 1971). In *Hypopachus* – another microhylid – a larval stage occurs in which ganglia and roots are found completely fused (SOKOL, 1975).

Although the information analyzed in this work is still very limited, the following conclusions are obtained:

(1) The prootic ganglion is not present in all neobatrachian larvae.

(2) In neobatrachian ontogeny, formation of the prootic ganglion can occur in two ways: in some species, formation of the ganglion precedes fusion of the trigeminal and facial roots; in others, fusion of the roots occurs first.

(3) There are interspecific heterochronies in the formation of the prootic ganglion.

(4) Analyses of the intracranial relation of the trigeminal and facial nerves during neobatrachian ontogeny will provide new information to clarify their phylogenetic relationships.

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Literature cited

De Beer, G. R., 1937. — *The development of the vertebrate skull* Oxford, Clarendon Press; reprint 1985, Chicago, Univ. Chicago Press. i-xlvi + 1-554, pl 143.
De Jongh, H. J., 1968 — Functional morphology of the jaws apparatus of larval and metamorphosing *Rana temporaria* L. *Neth. J. Zool.*, **18**: 1-103.
Duellman, W. E. & Trueb, L., 1985. — *Biology of amphibians*. New York, McGraw-Hill, "1986"; reprint 1994, Johns Hopkins University Press: i-xxi + 1-670.
Filipski, G. T. & Wilson, M H. V., 1984. — Sudan Black B as a nerve stain for whole cleared fishes. *Copeia*, **1984**: 204-208.
----- 1985. — Staining nerves in whole cleared amphibians and reptiles using Sudan Black B. *Copeia*, **1985**: 500-502.
Ford, L. S. & Cannatella, D C., 1993. The major clades of frogs *Herp. Mon*, 7: 94-117.
Goodrich, E. S., 1930. — *Studies on the structure and development of vertebrates*. Chicago, Univ. Chicago Press; reprint 1958, Dover Publications Inc.: i-lxix + 1-837.
Gosner, K L., 1960 A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, **16**: 183-190.
Jacobson, C M., 1968. — The development of the chondrocranium in two species of the Australian anuran genus *Pseudophryne* Fitzinger. *Aust. J. Zool.*, **16**: 1-16.
Laurent, R. F, 1986. — Sous-classe des Lissamphibiens (Lissamphibia) Systématique. *In*: P.-P. Grassé & M. Delsol (eds.), *Traité de zoologie*, tome **14**, *Batraciens*, fasc. 1 B, Paris, Masson. 594-797.
Nishikawa, K. C., 1987. — Staining amphibian peripheral nerves with Sudan Black B. progressive vs. regressive methods. *Copeia*, **1987**: 489-491.
Orton, J. L., 1953. — The systematics of vertebrate larvae. *Syst. Zool.*, **2**: 63-75.
Paterson, N. F., 1945. The skull of *Hymenochirus curtipes*. *Proc. zool. Soc. London*, **115** 327-354.
Plasota, K., 1974 — The development of the chondrocranium (neurocranium, and the mandibular and hyoid arches) in *Rana temporaria* L. and *Pelobates fuscus* (Laur.). *Zool. Pol.*, **24** 99-118.
Pusey, H K, 1938. — Structural changes in the anuran mandibular arch during metamorphosis, with reference to *Rana temporaria*. *Q. J. micr. Sci.*, **80** 479-465.
----- 1943 — On the head of the leiopelmid frog, *Ascaphus truei*. I The chondrocranium, jaws, arches, and muscles of a partly-grown larva. *Q. J. micr. Sci.*, **84**. 105-185.
Roček, Z, 1981. — Cranial anatomy of frogs of the family Pelobatidae Stannius, 1856, with outlines of their phylogeny and systematics. *Acta Univ. Carolinae, Biol.*: 1-164
Schlosser, G. & Roth, G., 1995. — Distribution of cranial and rostral spinal nerves in tadpoles of the frog *Discoglossus pictus* (Discoglossidae). *J Morph*, **226**: 189-212
Sokol, O. M., 1975. The phylogeny of anuran larvae: a new look. *Copeia*, **1975**: 1-23.
----- 1977 The free-swimming *Pipa* larvae, with a review of pipid larvae and pipid phylogeny (Anura: Pipidae). *J. Morph.*, **154**: 357-425.

----- 1981. — The larval chondrocranium of *Pelodytes punctatus*, with a review of tadpoles chondrocrania. *J. Morph.*, **169**: 161-183.

STARRETT, P. H., 1973. — Evolutionary patterns in larval morphology *In:* J L VIAL (ed.), *Evolutionary biology of the anurans*, Columbia, Univ. Missouri Press: 252-271.

STEPHENSON, N. G., 1950 — On the development of the chondrocranium and visceral arches of *Leiopelma archeyi*. *Trans. zool. Soc. London*, **27**: 203-253.

SWANEPOEL, J. H., 1970. — The ontogenesis of the chondrocranium and of the nasal sac of the microhylid frog *Breviceps adspersus pentheri* Werner. *Ann. Univ. Stell.*, **45** (A): 1-119.

VAN EEDEN, J A., 1951. — The development of the chondrocranium of *Ascaphus truei* Stejneger, with special reference to the relations of the palatoquadrate to the neurocranium. *Acta zool*, **32**: 1-136.

WASSERSUG, R. J., 1976. — A procedure for differential staining of cartilage and bone in whole formalin-fixed vertebrates. *Stain Technol.*, **51**: 131-134.

WESTHUIZEN, C M. V. D, 1961. — The development of the chondrocranium of *Heleophryne purcelli* Sclater with special reference to the palatoquadrate and the sound-conducting apparatus. *Acta zool.*, **42**: 1-72.

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APPENDIX 1
LIST OF SPECIMENS EXAMINED

FAMILY LEPTODACTYLIDAE

Ceratophrys cranwelli. — MCN 021: whole mounts stained for peripheral nerves of 20 specimens at stages 31-34 and 2 specimens at stage 42; transverse serial sections of 2 specimens at stage 33; whole mounts stained for bone and cartilage of 10 specimens at stages 31-34. FML 4534: whole mounts stained for bone and cartilage of 7 specimens at stages 40-46. FML 4573: dry skeleton of 1 adult specimen. FML 4574: dry skeleton of 1 adult specimen.

Lepidobatrachus llanensis. — FML 4678: whole mounts stained for peripheral nerves of 5 specimens at stages 31, 33, 35, 37 and 42; whole mounts stained for bone and cartilage of 4 specimens at stages 31, 33, 37 and 44. FML 0420: dry skeleton of 1 adult specimen. FML 1016: dry skeleton of 1 adult specimen. FML 5220: dry skeleton of 1 adult specimen. FML 5221: dry skeleton of 1 adult specimen. MCN 081: dissection of 1 adult specimen.

Physalaemus biligonigerus. — MCN 043: whole mounts stained for peripheral nerves of 22 specimens at stages 31-42; whole mounts stained for bone and cartilage of 10 specimens at stages 32-41. MCN 157: whole mounts stained for bone and cartilage of 4 adult specimens.

FAMILY HYLIDAE

Hyla pulchella andina. — MCN 024: whole mounts stained for peripheral nerves of 14 specimens at stages 31-42; whole mounts stained for bone and cartilage of 10 specimens at stages 33-39. MCN s/n: whole mounts stained for bone and cartilage of 2 adult specimens.

Phyllomedusa sauvagii — MCN 061: whole mounts stained for peripheral nerves of 18 specimens at stages 31-42; transverse serial sections of 2 specimens at stage 33; whole mounts stained for bone and cartilage of 7 specimens at stages 33-38. FML 3823: whole mounts stained for bone and cartilage of 2 adult specimens.
Scinax fuscovaria. — MCN 027: whole mounts stained for peripheral nerves of 19 specimens at stages 31-41; whole mounts stained for bone and cartilage of 7 specimens at stages 33-38. MCN 072: whole mounts stained for bone and cartilage of 2 adult specimens.

FAMILY MICROHYLIDAE

Dermatonotus muelleri. — FML 4694: whole mounts stained for peripheral nerves of 6 specimens at stages 27, 29, 30, 33, 35 and 37; whole mounts stained for bone and cartilage of 3 specimens at stages 33-35. MCN 056: whole mounts stained for peripheral nerves of 5 specimens at stages 31-36; transverse serial sections of 3 specimens at stage 33; whole mounts stained for bone and cartilage of 7 specimens at stages 35-41 FML 1074: whole mounts stained for bone and cartilage of 1 adult specimen.

Description of the tadpole of *Bufo kisoloensis*

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The tadpole of *Bufo kisoloensis* from the Impenetrable Forest of extreme southwestern Uganda is described and compared with the tadpole of *Bufo maculatus*. The morphology of the *B. kisoloensis* tadpole reflects the conservative morphology of the tadpoles in this genus but can be distinguished by a set of internal buccal characters, particularly the number and arrangement of the papillae.

Introduction

African tadpoles are relatively poorly known, especially those in the central and eastern parts of the continent. Tadpoles of 18 of 31 species of bufonids in central and southern Africa have been described (Wager, 1965; Van Dijk, 1971, Channing, 1972, 1973, 1978), and although buccal features provide useful taxonomic characters, these structures have been described for very few African *Bufo* tadpoles.

Bufo kisoloensis is a large (males to 80 mm; females to more than 90 mm), high-altitude species known from above 2000 m in the highlands associated with the Albertine Rift of Kenya west of the Great Rift, northern Malawi and adjacent southern Tanzania (Tandy & Keith, 1972). A member of the *B. regularis* complex, it was described as a subspecies of *B. regularis* (Loveridge, 1932) and elevated to specific rank by Laurent (1952). Schmidt & Inger (1959) confirmed the specific status and provided additional diagnostic characters.

The bright chrome yellow color of breeding males of *B. kisoloensis* is unique among African *Bufo*. Except during breeding periods, males are cryptically colored like females. Sexual dichromatism (which may be permanent) also occurs in several other species of *Bufo*, such as *Bufo canorus* of California, *B. luetkenii* of Nicaragua and Costa Rica, the possibly extinct *B. periglenes* of Costa Rica, and *B. peripatetes* of Panama (Savage, 1966; Villa, 1972; Savage & Donnelly, 1992).

The *B. kisoloensis* tadpoles were collected from a small stream (altitude 1700 m) within an area dominated by *Cyathea* and *Lobelia*. Adults in breeding condition and amplecting pairs were found in various habitats in the forest ranging from disturbed areas, through simple rush-sedge swamps, to complex *Acanthocleista-Cyperus-Thelypteris-Begonia* swamps (Drewes & Vindum, 1994). The identification of the tadpoles is based on

B. kisoloensis being the only bufonid in the forest proper (Drewes & Vindum, 1994). *Bufo maculatus*, considered by Tandy & Keith (1972) to be in a group of its own, occurs at lower elevations in the savannas of west and east Africa south to northeastern Kwazulu-Natal and breeds in low-elevation, disturbed habitats on the periphery of the Impenetrable Forest.

Description

Staging follows the table of Gosner (1960), buccal features (stained with Fast Green) are described with the terminology of Wassersug (1976, 1980), descriptive characters and nomenclature are based on Van Dijk (1966), and the keratodont formula follows the recommendations of Dubois (1995).

The description is based on one tadpole (stage 34, 19 mm total length) from over 90 tadpoles (California Academy of Sciences 180664) collected from the Impenetrable Forest Reserve in extreme southwestern Uganda by R. C. Drewes, H. W. Greene, J. P. O'Brien and J. V. Vindum on 5 November 1990. Comparisons with four other tadpoles from the sample that ranged from stages 31 to 41 and from 18 to 20 mm total length showed no appreciable differences in the characters we examined.

External features

The tail length (fig. 1) is 51 % of the total length, and tail height is slightly greater than body height. The tail tip is bluntly rounded. The maximum height of the dorsal fin occurs midway along the tail. The tail is euthyoural with the extrapolated axis passing through the eye. The basal height of the caudal muscle is less than half (44 %) the height of the body.

The kidney-shaped nostrils with a slightly raised medial projection on the proximal margin are positioned slightly closer to the eye than to the snout. The nasal passages are visible dorsolaterally. The ratio nostril width/internarial distance is 0.15. The orbitonasal line and pineal spot are not visible. The ratio rostronasal distance (measured between closest margins)/orbitonasal distance is 1.6.

The eyes are dorsolateral, and the extra-ocular proportion (head width minus distance between the lateral limits of the eyes/distance between the lateral limits of the eyes) is 0.48. The spiracle is sinistral, visible dorsally, and situated 60 % posteriorly along the body; the flattened aperture is oval and visible laterally. The medial vent is situated on the ventral margin of the fin.

The ventral oral disc is not visible dorsally and is 75 % of the width of the head at the level of the disc. A single row of papillae is present laterally on the disc margin, and the mental gap is half the width of the disc. The suprarostrodont is finely serrated, edged with black and grading to a dark brown on the basal half. The widely V-shaped infrarostrodont is also finely serrated, and the distal half is deeply pigmented in brown. The keratodont formula is 1:1+1/3 (fig. 2).

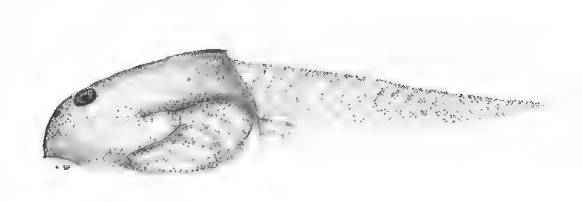


Fig. 1 — Left lateral view of a tadpole of *Bufo kisoloensis* (California Academy of Sciences 180664; stage 34, 19 mm total length).

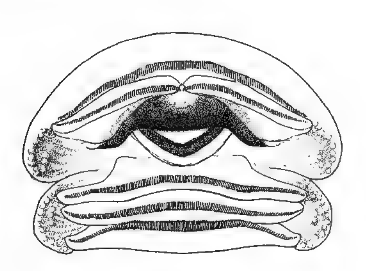


Fig. 2. — Mouthparts of a tadpole of *Bufo kisoloensis*.

Tab. 1. - Comparison of the buccal anatomy of *Bufo maculatus* and *Bufo kisoloensis* tadpoles.

Bufo maculatus	*Bufo kisoloensis*
1 postnarial lateral papilla	2 postnarial lateral papillae, lateral one forms lateral margin of median ridge
Inner pair of postnarial arena papillae simple	Inner pair of postnarial arena papillae notched
4 pairs of lateral papillae in buccal roof arena	3 pairs of lateral papillae in buccal roof arena
A very large pustulation on either side of the anterior border of the velum	No such large pustulations
5-6 major papillae on each side of buccal floor arena	13 major papillae on each side of buccal floor arena

Dorsally the tadpoles are uniform dark brown rather than black as is common in many species of *Bufo*, and the venter a uniform lighter brown. The tail is evenly pigmented almost to the ventral margin over the anterior two thirds. The dorsal fin is uniformly pigmented, while the ventral fin is only pigmented anteriorly. Some tadpoles have a diffuse mottling on the ventral fin.

Internal features

The internal nares are anteriorly convergent at about 50° to the midline. A pair of large, finely scalloped postnarial papillae form the lateral margin of the postnarial arena. This arena has a few small pustulations and two pairs of papillae. The median ridge is defined by a large central papilla and an elongated lateral papilla on each side. The buccal roof arena has a number of minute pustulations and three long lateral roof papillae on each side. A rounded area on the posterior midline stains darkly.

The buccal floor has an anterior raised lingual pad flanked by trilobed infralabial papillae and three smaller lingual papillae. The arena is flat with regularly-spaced minute pustulations and flanked by 13 elongated papillae on each side.

COMPARISONS AMONG AFRICAN BUFONID TADPOLES

The head ornaments of the tadpoles of the bufonid genera *Mertensophryne*, *Schismaderma* and *Stephopaedes* make them quite distinctive, but tadpoles of *Bufo* are quite uniform in morphology and size. Although internal buccal characters provide good characters for species identification, very little is presently known about these features in African *Bufo*. *Bufo maculatus* is the only other bufonid occurring near the Impenetrable Forest. The internal buccal anatomy of *B. kisoloensis* differs from that of *B. maculatus* (LAMBIRIS, 1994, as *Bufo pusillus*) as listed in tab. 1. Although all known *Bufo* tadpoles have similar overall morphology, it appears that buccal characters are diagnostic at the species level. As more tadpoles become known, it will be possible to review the internal buccal anatomy for the members of the genus in Africa.

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LITERATURE CITED

CHANNING, A., 1972. — A description of *Bufo pusillus* tadpoles (Anura: Bufonidae). *Ann. Natal Mus.*, **21**: 509-511.

----- 1973 — A description of *Bufo pardalis* tadpoles (Anura: Bufonidae). *Zool Afr.*, **8**: 153-156.

----- 1978. — A new bufonid genus (Amphibia. Anura) from Rhodesia. *Herpetologica*, **34**. 394-397.

DREWES, R. C. & VINDUM, J. V., 1994. — Amphibians of the Impenetrable Forest, southwest Uganda *J. afr. Zool.*, **108**: 55-70.

DUBOIS, A., 1995 — Keratodont formulae in anuran tadpoles: proposals for a standardization. *J. zool. Syst. Evol. Res.*, **33**: I-XV.

GOSNER, K. L., 1960. — A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, **16**: 183-190.

LAMBIRIS, A. J. L., 1994. — Laryngeal and buccopharyngeal morphology of some South African Bufonidae. new data sets for anuran taxonomy *Ann Natal Mus.*, 35: 261-307.

LAURENT, R. F., 1952. — *Bufo kisoloensis* Loveridge and *Chamaeleo ituriensis* Schmidt revived. *Herpetologica*, 8: 53-55.

LOVERIDGE, A., 1932. — Eight new toads of the genus *Bufo* from east and central Africa *Occ. Pap Boston Soc. nat. Hist*, **8**: 43-54.

SAVAGE, J. M., 1966. — An extraordinary new toad (*Bufo*) from Costa Rica. *Rev. Biol. trop.*, **14**: 153-167.

SAVAGE, J. M. & DONNELLY, M., 1992 — The second collection of, and variation in, the rare neotropical toad, *Bufo peripatetes*. *J. Herp.*, **26**: 72-74.

SCHMIDT, K. P. & INGER, R. F., 1959. — Explorations du Parc National de l'Upemba Amphibians exclusive of the genera *Afrixalus* and *Hyperolius Expl. Parc natn Upemba, Miss. G. F. De Witte*, **56**: 1-256.

TANDY, M. & KEITH, R., 1972. — *Bufo* of Africa. *In*: W. F. BLAIR (ed.), *Evolution in the genus* Bufo, Austin, University of Texas Press: 119-170.

VAN DIJK, D. E., 1966. — Systematic and field keys to the families, genera and described species of southern African tadpoles. *Ann. Natal Mus*, **18**: 231-286.

----- 1971. — A further contribution to the systematics of southern African anuran tadpoles — the genus *Bufo*. *Ann. Natal Mus*, **21**: 71-76.

VILLA, J, 1972. — *Anfibios de Nicaragua*. Managua, Inst. Geogr Nac. & Banco Central de Nicaragua: 1-216.

WAGER, V. A, 1965 — *The frogs of South Africa*. Cape Town, Purnell & Sons: 1-242.

WASSERSUG, R. J., 1976. Internal oral features in *Hyla regilla* (Anura: Hylidae) larvae: an ontogenetic study. *Occ. Pap. Mus. nat. Hist. Univ. Kansas*, **49**: 1-24.

----- 1980. — Internal oral features of larvae from eight anuran families: functional, systematic, evolutionary and ecological considerations. *Misc. Publ. Univ. Kansas Mus. nat. Hist.*, **68**: 1-146.

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La larva de *Melanophryniscus rubriventris rubriventris* (Vellard, 1947) (Anura, Bufonidae)

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Melanophryniscus rubriventris rubriventris **larvae are described based on 18 individuals in stages 31-34 of Gosner's (1960) developmental table. They are similar to other tadpoles of *Melanophryniscus* in terms of overall morphology, but differ from *Melanophryniscus moreirae*, *M. orejasmirandai* and *M. sanmartini* in the formula of keratodont rows, from *M. stelzneri montevidensis* in characters of the proctodeal tube and spiraculum, and from *M. stelzneri stelzneri* in characters of the spiraculum and size of the oral disc. No larval information is available regarding the remaining taxa of the genus.**

Introducción

Melanophryniscus rubriventris es una especie característica de las selvas de montaña del noroeste argentino y sur de Bolivia, en la que se reconocen tres subespecies: la nominal, descripta por Vellard (1947), registrada en los departamentos Orán (Salta) y Ledesma y Valle Grande (Jujuy) y *M. r. subconcolor* y *M. r. toldosensis*, descriptas por Laurent (1973) para las regiones de Tiraxi (Jujuy) y Los Toldos (Salta), respectivamente.

Como parte de un estudio mayor sobre la batracofauna de los Yungas comenzamos una serie de análisis sobre el modo de reproducción y desarrollo de *Melanophryniscus rubriventris rubriventris* en el Parque Nacional Calilegua, y en esta contribución describimos los estadios larvales de dicha subespecie.

Material y métodos

Se estudiaron larvas de *Melanophryniscus rubriventris rubriventris* en estadios comparables a 31-34 de Gosner (1960). Las mismas, fijadas y conservadas en formol 10 %, fueron descriptas siguiendo las pautas establecidas por Lavilla (1983) y medidas según lo establecido en dicho trabajo y en Lavilla & Scrocchi (1986). El largo total fue

Tab 1. - Medidas de una serie de 18 larvas de *Melanophryniscus rubriventris rubriventris* en estadios 31 a 34 de la tabla de Gosner (1960). *x*: promedio; *s*: desviación estándard.

Medida	Rango	*x*	*s*
LT: longitud total	14,5 - 18,2	16,7	1,16
LC: longitud del cuerpo	5,7 - 6,8	6,3	0,33
LCo: longitud de la cola	8,1 - 11,4	10,3	0,94
AM: ancho máximo del cuerpo	4,1 - 4,9	4,5	0,24
AO: ancho del cuerpo a nivel de los ojos	3,4 - 4,3	3,9	0,27
AOn: ancho del cuerpo a nivel de los orificios nasales	2,4 - 3,3	3,0	0,25
HM: altura máxima del cuerpo	3,3 - 4,3	3,7	0,30
HMU: altura de los músculos de la cola	1,2 - 1,5	1,35	0,12
HA: altura aletas	3,2 - 4,0	3,7	0,20
DRE: distancia rostro-espiráculo	3,9 - 5,0	4,4	0,28
FN: distancia frontonasal	1,1 - 1,3	1,16	0,11
NO: distancia naso-ocular	0,3 - 0,4	0,38	0,04
IN: distancia intranasal	0,7 - 0,9	0,81	0,07
IO: distancia intraocular	1,0 - 1,3	1,13	0,08
EN: distancia extranasal	1,2 - 1,5	1,37	0,09
EO: distancia extraocular	2,2 - 2,6	2,36	0,12
O: diámetro del ojo	0,6 - 0,8	0,69	0,07
ON: diámetro del orificio nasal	0,2 - 0,4	0,29	0,05
DO: ancho del disco oral	1,8 - 2,1	1,99	0,12
CR: ancho del claro rostral	1,2 - 1,7	1,46	0,13
CM: ancho del claro mental	1,1 - 1,4	1,22	0,09

tomado utilizando un calibre con precisión 0,02 mm y las restantes medidas con ocular micrométrico bajo lupa binocular.

El material analizado (18 ejemplares) forma parte de un lote mayor depositado en la colección del Instituto de Herpetología de la Fundación Miguel Lillo (FML) bajo el número 04731, proveniente de Abra de Cañas, aproximadamente a 23°35'S 64°50'W y a 1700 m s.n.m., en el Departamento Ledesma, Jujuy, Argentina, colectados el 2 de enero de 1991. La identificación se realizó en base a una serie completa de desarrollo.

Los valores señalados en el texto (en milímetros) corresponden al promedio y a la desviación estándar (*s*), mientras que los rangos se presentan en la tab. 1.

RESULTADOS

Las larvas de *Melanophryniscus rubriventris rubriventris* fueron colectadas en charcos temporarios de menos de 20 cm de profundidad, con sustrato arcilloso y sin vegetación en una región de selva de montaña. La subespecie se reproduce después de fuertes precipitaciones en grandes agrupaciones, observándose gran cantidad de amplexos múltiples y búsqueda activa por parte de los machos. Observaciones realizadas han mostrado que existen variaciones en la puesta de huevos según los sitios escogidos, que van desde huevos colocados individualmente y dispersos en el fondo del agua a huevos agrupados en masas y adheridos a la vegetación sumergida. *Melanophryniscus rubriventris rubriventris* es el único taxon del género registrado en selvas de montaña de Argentina, y los caracteres de puesta lo diferencian de los restantes bufonidos de la región (*Bufo paracnemis, B. arenarum, B. rumbolli* y *B. gallardoi*), quienes colocan sus huevos en cordones gelatinosos.

En estadios 31-34 de GOSNER (1960), las larvas de *Melanophryniscus rubriventris rubriventris* (fig. 1) presentan una longitud total entre 14,5 y 18,2 mm (n = 18 ejemplares); el cuerpo es deprimido (la altura es menor que el ancho = 0,82, s = 0,05) y su aspecto dorsal muestra la región anterior a los ojos subtriangular, en tanto que la porción posterior del cuerpo presenta márgenes subparalelos a convergentes hacia atrás, no existiendo constricciones notables. El ancho máximo se ubica inmediatamente por detrás de los ojos, todavía sobre la región cefálica del cuerpo. El hocico es redondeado en vistas dorsal y lateral, las regiones gular y branquial son plano-cóncavas y la región abdominal es plano-convexa.

El disco oral, de tamaño pequeño (ancho del disco oral/ancho máximo del cuerpo = 0,44; s = 0,01) es de posición subterminal ventral. Los márgenes anterior y posterior son lisos y lateralmente presentan aspecto dentado, estando divididos por una hendidura angular a cada lado. La hilera marginal de papilas esta interrumpida por un claro rostral grande (ancho del claro rostral/ancho del disco oral = 0,72; s = 0,05) y un claro mental mediano (ancho del claro rostral/ancho del disco oral = 0,61; s = 0,04). Las papilas marginales, limitadas a las regiones laterales del disco, son simples y se disponen en una hilera única tanto supra como infraangularmente; pueden existir o no papilas intramarginales en la región supraangular, ubicadas próximas a las de la hilera marginal, de las que se diferencian por su tamaño mayor. También pueden aparecer papilas aisladas en la región angular.

El suprarostrodonte (pico córneo superior) es más ancho que alto y su margen libre es uniformemente cóncavo, presentando aserraduras subtriangulares agudas; sólo está queratinizada y pigmentada (castaño oscuro) su mitad distal. El infrarostrodonte (pico córneo inferior) tiene forma de V muy abierta, está queratinizado y pigmentado sólo en el tercio distal y posee aserraduras similares a las del suprarostrodonte. Los queratodontes se disponen según fórmula 2/3, y cada denticulo es simple y con extremo romo.

Los orificios nasales son circulares y presentan un reborde bajo; no están protruídos, carecen de inflexiones y presentan una proyección poco notable en el margen interno. Se abren a nivel de la superficie del cuerpo y están ubicados más cerca del ojo que del extremo del hocico (distancia frontonasal/distancia naso-ocular = 2,90; s = 0,56), en posición

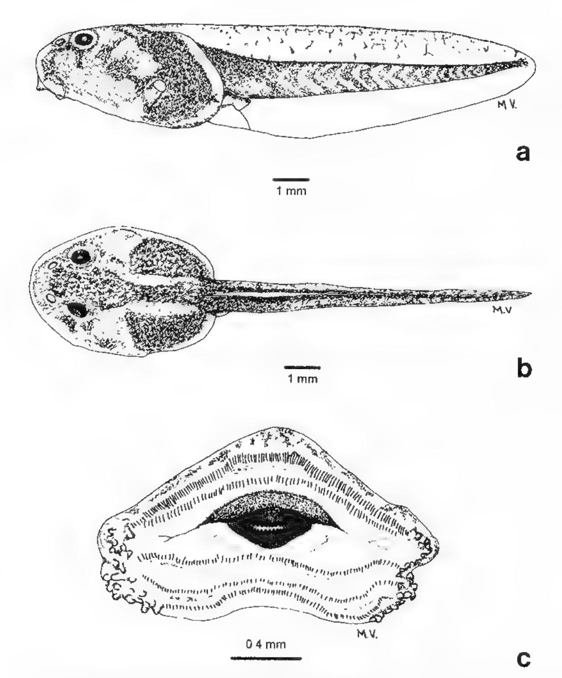


Fig. 1. — Larva de *Melanophryniscus rubriventris rubriventris*, estadio 31 de Gosner (1960). (a) Vista lateral. (b) Vista dorsal. (c) Disco oral.

dorsolateral (distancia extranasal/ancho del cuerpo a nivel de los ojos = 0,48; s = 0,05). Son visibles dorsal, frontal y lateralmente, y la pigmentación alrededor de ellos es levemente más oscura que la superficie circundante. El pasaje nasal es invisible.

Los ojos son de tamaño mediano (diámetro del ojo/ancho del cuerpo a nivel de los ojos = 0,18; s = 0,01), están ubicados dorsolateralmente (distancia extraocular/ancho del cuerpo a nivel de los ojos = 0,61; s = 0,04) y la línea orbitonasal es invisible.

El espiráculo es único, izquierdo y visible dorsalmente. El tubo espiracular es corto y no está proyectado, por lo que su abertura, subcircular, abre a nivel de la superficie general del cuerpo en posición ventrolateral. Está, además, desplazado hacia la mitad posterior del cuerpo (distancia rostro-espiráculo/longitud cuerpo = 0,69; s = 0,03).

El tubo proctodeal es de aspecto cónico, más ancho en la base que en el extremo libre y abre a la derecha de la aleta ventral. Un carácter peculiar observado es que los pliegues del intestino son laxos y el asa intestinal está desplazada hacia la izquierda del cuerpo, ubicándose muy próxima a la abertura del espiráculo.

La cola es mediana (longitud cola/longitud total = 0,62; s = 0,02), y su altura es variable con relación a la altura del cuerpo (altura aletas caudales/altura máxima del cuerpo = 0,91-1,06); las aletas dorsal y ventral poseen el margen libre uniformemente curvado y el extremo es ampliamente redondeado. El nacimiento de la aleta dorsal está levemente desplazado sobre el cuerpo, mientras que el nacimiento de la aleta ventral está asociado al tubo proctodeal, el eje de la cola es recto (eutiural).

Coloración en fijador (formol 10 %). — La piel del cuerpo es translúcida a transparente; en vista dorsal la región cefálica es de color castaño mediano y la región posterior algo más oscura, observándose el mismo patrón lateralmente. Ventralmente son transparentes, pudiendo haber puntos agrupados en el área central de la región branquial; una fascia melánica recubre parcialmente a la región abdominal, pero a pesar de ello el intestino es claramente visible. El tubo proctodeal presenta una estrecha banda de pigmento a nivel de la abertura, muy poco notable en algunos ejemplares El resto de su superficie ventral carece de manchas. La musculatura caudal es castaña, con áreas no pigmentadas irregulares, más abundantes en la mitad posterior; toda la superficie ventral de la musculatura hipaxial carece de pigmento. La aleta dorsal es translúcida, con manchas alargadas y estrechas distribuidas irregularmente en toda su superficie; la aleta ventral es translúcida y generalmente inmaculada, aunque en algunos ejemplares pueden aparecer pequeñas manchas de distribución irregular.

DISCUSIÓN

La información disponible sobre las larvas de *Melanophryniscus* es proporcionalmente escasa; de los catorce taxa que componen el género, se han descripto las larvas de *M. moreirae* (AHL, 1938; BOKERMANN, 1967; STARRETT, 1967), *M. orejasmirandai* (PRIGIONI & LANGONE, 1990), *M. sanmartini* (PRIGIONI & ARRIETA, 1992), *M. stelzneri montevidensis* (GARRIDO-YRIGARAY, 1989) y *M. stelzneri stelzneri* (FERNANDEZ, 1926, y una síntesis en CEI, 1980). Por su parte, MCDIARMID (1971) presentó una diagnosis en base a

caracteres larvales, aunque algunos taxa se apartan de ella en uno o más caracteres (vide infra).

Ninguna de las descripciones disponibles hace mención a caracteres del intestino, por lo que resaltamos la disposición particular del asa intestinal que se presenta en todas las larvas examinadas de *M. rubriventris rubriventris*.

Las larvas de *Melanophryniscus rubriventris rubriventris* cumplen con las características genéricas (McDiarmid, 1971), presentando espiráculo único e izquierdo, tubo proctodeal que abre a la derecha de la aleta caudal, fórmula dentaria 2/3, papilas labiales marginales en hilera simple y de posición lateral, y disco oral de posición subterminal ventral.

Difieren de las larvas de *M. moreirae*, *M. orejasmirandai* y *M. sanmartini* por el modo en que se disponen las hileras de queratodontes (1:1 + 1/1 + 1:2, 2/1 + 1:2 y 1 + 1/1 + 1:2 respectivamente, contra 2/3 en *M. r. rubriventris*).

Difiere de *M. s. montevidensis* por caracteres del tubo proctodeal y del espiráculo: el primero abre en la linea media (contra abertura dextral en *M. r. rubriventris*) y el segundo en la mitad del cuerpo (contra abertura en el tercio posterior).

Algunas diferencias con larvas de *M. stelzneri stelzneri* pueden ser inferidas a partir del trabajo de Fernandez (1926). Se destacan: ancho del disco oral 1 mm (contra 1,8 a 2,15 en *M. r. rubriventris*) y espiráculo ubicado en el tercio medio (contra espiráculo ubicado en el tercio posterior).

Es necesario resaltar que estas comparaciones deben ser tomadas como preliminares, dado que una constante en todas las descripciones analizadas es que están basadas en un sólo ejemplar, y los estadios analizados por los diversos autores son variables o no han sido definidos.

Resumen

Describimos las larvas de *Melanophryniscus rubriventris rubriventris* en base a 18 individuos que se encontraban en estadios 31-34 de Gosner (1960). Por sus caracteres generales son similares a las restantes larvas conocidas para el género *Melanophryniscus*, pero difieren de *Melanophryniscus moreirae*, *M. orejasmirandai* y *M. sanmartini* en el número de hileras de queratodontes; difieren de *M. stelzneri montevidensis* por caracteres del tubo proctodeal y del espiráculo y difieren de *M. stelzneri stelzneri* por caracteres del espiráculo y el tamaño del disco oral. No existe información disponible para las larvas de los restantes taxa del género.

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LITERATURA CITADA

AHL, E., 1938. — Über die Kaulquappe des *Dendrophryniscus moreirae* Miranda-Ribeiro. *Zool. Anz.*, **124** (5/6): 158-159.

BOKERMANN, W C. A., 1967. — Observaçoes sobre *Melanophryniscus moreirae* (Mir. Rib.) (Amphibia — Brachycephalidae). *An. Acad. bras. Ci.*, **39** (2): 301-306.

CEI, J. M., 1980. — Amphibians of Argentina. *Monit. zool. ital*, (n.s), Mon **2**: i-xii + 1-609.

FERNANDEZ, K., 1926 — Sobre la biología y reproducción de batracios argentinos. Segunda Parte. *Bol. Acad. nac. Ci. Córdoba*, **29**. 271-320, 4 lám.

GARRIDO-YRIGARAY, R R, 1989 — Descripción de la larva de *Melanophryniscus stelzneri montevidensis* (Philippi, 1902). *Bol. Soc. zool. Uruguay*, (2), **5**: 7-8.

GOSNER, K L., 1960 — A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, **16**: 183-190.

LAURENT, R. F., 1973. — Variación geográfica de *Melanophryniscus rubriventris* (Vellard). *Acta zool. lilloana*, **26** (23): 319-334.

LAVILLA, E. O., 1983. — *Sistemática de larvas de Telmatobiinae (Anura: Leptodactylidae)*. Tesis inédita, Facultad de Ciencias Naturales, Universidad Nacional de Tucumán i-v + 1-354.

LAVILLA, E. O. & SCROCCHI, G. J., 1986. — Morfometría larval de los géneros de Telmatobiinae (Anura Leptodactylidae) de Argentina y Chile. *Physis*, (B), **44** (106). 39-43.

MCDIARMID, R. W., 1971. — Comparative morphology and evolution of frogs of the neotropical genera *Atelopus*, *Dendrophryniscus*, *Melanophryniscus* and *Oreophrynella*. *Bull Los Angeles Co Mus. nat. Hist.*, **12**: 1-66, 1 lám

PRIGIONI, C. M. & ARRIETA, D., 1992. — Descripción de la larva de *Melanophryniscus sanmartini* Klappenbach, 1968 (Amphibia: Anura: Bufonidae). *Bol. Soc. zool. Uruguay*, (2), **7** 57-58.

PRIGIONI, C. M. & LANGONE, J. A., 1990. — Descripción de la larva de *Melanophryniscus orejasmirandai* Prigioni & Langone, 1986 (Amphibia, Anura, Bufonidae). *Com. Mus nac Hist. nat. Montevideo*, **12** (173): 1-9.

STARRETT, P., 1967. — Observations on the life history of frogs of the family Atelopodidae. *Herpetologica*, **23** (3): 195-204.

VELLARD, J, 1947. — Un nuevo batracio del norte argentino. *Acta zool. lilloana*, **4**: 115-119.

Corresponding editor: Ronald G. ALTIG.

Alytes, 1997, **15** (1): 26-36.

Microanatomy of the buccal apparatus and oral cavity of *Hyla minuta* Peters, 1872 larvae (Anura, Hylidae), with data on feeding habits

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Histological and SEM observations of the buccal structures and reduced mouthparts of the tadpole of *Hyla minuta* revealed new anatomical features. There are few columns of cells that produce labial teeth in rows A-1 and P-2, and each column has only a few cells that produce teeth. The labial teeth are lightly pigmented, short and with 3-5 short cusps. Tooth development seems to be abbreviated and not continuous during ontogeny.

Tooth densities vary among rows, and tooth rows A-1, P-1 and P-2 may be incomplete or A-1 and P-2 may be absent. The number of conical cells in different stages of keratinization in the cellular columns of the jaw sheaths also varies.

Buccal roof arena papillae are sparse and tall, there are no lingual papillae, the ventral velum has reduced marginal projections, median and lateral ridges are present, and there are secretory pits on the dorsal velum and posterior buccal roof. Based on intestinal contents (i.e., periphytic algae, pieces of filamentous algae and cyperaceous plants including meristematic tissue, and free starch granules), these tadpoles have a broad, herbivorous macrophagous diet that appears to be harvested primarily by the jaw sheaths.

INTRODUCTION

The oral apparatus of anuran larvae usually consists of an oral disc with keratinized jaw sheaths and labial teeth positioned on transverse tooth ridges. The jaw sheaths are usually strongly pigmented and have a serrated edge, and labial teeth usually have cusps on the head. Histological features (e. g., *Alytes obstetricans*: BEAUMONT & DEUNFF, 1959; BOURGES & BACHELERIE, 1974, *Discoglossus pictus*: DEUNFF & BEAUMONT, 1959; *Rana pipiens*: CHENG, 1964, fide KUANG, 1975, LUCKENBILL, 1965) and development of these mouthparts (e.g., *Rana pipiens*: LUCKENBILL, 1965; KUANG, 1975; *Bufo arenarum*: FIORITO DE LOPEZ & ECHEVERRÍA, 1984, 1989) have been reported for a number of species with a labial tooth row formula of 2/3. This formula is extremely common in anuran tadpoles in many taxa and several ecomorphological guilds (ALTIG & JOHNSTON, 1989), but there are two general patterns that deviate from the norm: increases in tooth row number in lotic

tadpoles and decreases in tooth rows in several groups. Tooth row reductions accompanied by various other modifications of the oral apparatus are assumed to have occurred independently in four groups of small South American *Hyla*: *leucophyllata*, *microcephala*, *minuta* and *parviceps*. The pattern of reduction also varies among groups. *Hyla microps* larvae have neither labial ridges nor labial teeth (HEYER et al., 1990). In *Hyla nana*, labial teeth are absent, and the mouth is modified into a tube (LAVILLA, 1990). *Hyla sarayacuensis* tadpoles have labial ridges but lack teeth (ALTIG & JOHNSTON, 1989).

In an attempt to supply the comparative morphological information required to evaluate the assumption that tooth row reductions reflect a change in feeding mode compared with more typical tadpoles, I report the oral and buccopharyngeal anatomy of the tadpole of *Hyla minuta*.

MATERIAL AND METHODS

Hyla minuta tadpoles were obtained from semipermanent pools (maximum depth 40 cm) with rooted vegetation near the Iguazú River, Iguazú National Park, Provincia Misiones, Argentina. Samples were obtained from April to December (autumn to summer) in 1989-1993. Some tadpoles were reared through metamorphosis and preserved (SBM 38) to ascertain identification. Ten tadpoles were examined histologically, eight were observed with SEM, and two were dissected for camera lucida drawings. All specimens (stages 25-39) were staged by the table of GOSNER (1960), preserved in 10 % formalin when they were captured, and stored in the dark in 5 % formalin.

For light microscopy, the tadpole body was dehydrated, cleared, and embedded in paraffin (56-58°C), and serial sagittal and longitudinal sections cut at 4 and 7 µm were stained with Masson's trichrome (MARTOJA & MARTOJA-PIERSON, 1970). For scanning electron microscopy, the oral disc and buccal structures were critical-point dried and coated with gold-palladium. These specimens also were drawn with a camera lucida, and a video recording of some of the SEM observations is kept in the MEB-VIDEO (1993-94) collection. Lengths of labial tooth rows P-1 and P-2 were taken in conjunction with the SEM observation, according to ECHEVERRÍA's (1992) proposal. Drawings of the buccal structures were made with a camera lucida from specimens in stages 30 and 37.

Terminology follows ALTIG (1970) and DEUNFF & BEAUMONT (1959) for oral disc and tooth features, and VIERTEL (1982) for internal oral features. The descriptions of the histological features are based on FIORITO DE LOPEZ & ECHEVERRÍA (1989).

Pieces of the anterior intestine were dissected from two specimens at stages 25 and 32 for qualitative examination of the intestinal content by histological sections and with SEM.

RESULTS

SEM OBSERVATIONS

The oral disc has a single row of large marginal papillae along the lateral and ventral sides, and submarginal papillae are absent (fig. 1A). There are one upper and two lower labial ridges, and teeth are present on P-1 but often absent in A-1 and P-2. Row P-1 extends almost across the transverse diameter of the disc, but teeth in P-2 are frequently restricted to the medial area. If teeth are present in row A-1, the labial teeth are less dense (ca. 10-15 µm apart) than in P-1 and P-2. Individual teeth have a short head and no neck with modal total lengths of 15 µm and modal widths of 9 µm in P-1. There are 3 or 5 short, sharp, sharply angled cusps on each tooth, and the short sheath is tightly anchored to the soft tissue of the tooth ridge. In different rows (A-1 and P-1) and in different clutches, labial teeth morphology may vary (figs. 1B-C). The wide jaw sheaths have regular, conical serrations on the cutting edge (figs. 1A, D). The total modal length of these serrations is 20 µm, and modal width is 15 µm.

BUCCAL FLOOR AND ROOF FEATURES (FIGS. 2A-B)

The floor of the buccal cavity is triangular, and the branchial traps beneath the buccal floor are not evident. Two infralabial papillae (IP) present behind the jaw sheath are compressed and have a flat base and low projections on the free edge. Between these, a pair of prelingual papillae arranged in a transverse row have a rugose or slightly denticulate edge (fig. 3). The tongue anlage is large and lingual papillae are absent. The buccal floor arena is very broad but not well defined and has a densely pustulate surface, and no lateral papillae outline the arena. The complex prepocket papillae arise from a common base, and the obliquely oriented buccal pockets are ovoid with the long axis directed anteromedially. There are 1-2 pairs of medial projections on the posterior edge of the velum, and large secretory pits occur along the velum.

The roof of the buccal cavity is shaped like an equilateral triangle. The large, elongate internal nares are oriented obliquely, and the walls have a smooth narial valve. Postnarial papillae are absent. The postnarial arena contains (stages 25-36) a triangular pre-median ridge with three primary projections; the middle one is the largest (fig. 4A). By stage 37, this ridge is shaped like an inverted V (fig. 2B). Pustulations are present in the postnarial arena, and the lateral ridge is composed of a pair of long, simple and conical papillae. The anterior papillae have smooth or rugose edges, and the posterior ones are the tallest and are compressed laterally.

The triangular or crescent-shaped median ridge has small projections medially, and the poorly defined buccal roof arena has a dense field of pustules and a pair of long, conical, smooth marginal arena papillae on the posterior side of the arena. The glandular zone is straight, transverse, and bears secretory pits. The dorsal velum and marginal projections are absent (fig. 4B).

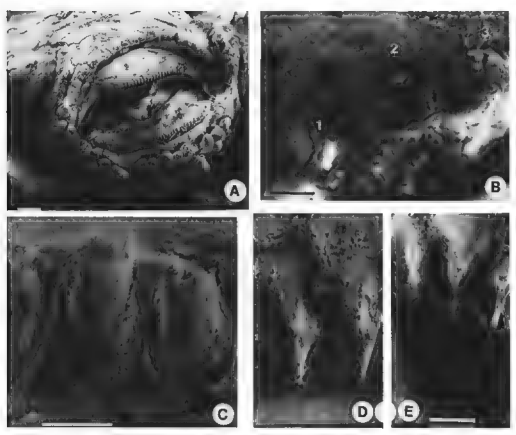


FIG. 1. — (A) SEM micrograph of the oral apparatus of *Hyla minuta*, stage 25. Scale line 100 µm. (B) Three labial teeth (1, 2, 3) emerging in row A-1, stage 25. Scale line: 10 µm. (C) Labial teeth of row P-1, stage 25 Scale line: 10 µm (D) Front view of upper jaw serrations, stage 25 (E) Rear view of same as in (D). Scale line: 10 µm.

LIGHT MICROSCOPE OBSERVATIONS

Sagittal sections show that the lower jaw sheath is longer than wide, and the infrarostral cartilage is subcircular in section. The suprarostral cartilage is long and narrow, and the upper jaw sheath is deeply convex and thicker than the lower sheath (fig. 5A). The upper and lower jaw cartilages are covered with a modified stratified epithelial tissue that gives rise to the cells that form the jaw sheaths. The core of the epithelium consists of several rows of cellular columns that contain several morphological cell types (figs. 5B-C): basal columnar cells lying over the basal membrane, flattened precone cells with basophilic cytoplasm, and cone cells in different stages of progressive keratinization from the proximal to distal end of the column. A layer of stratified epithelial tissue forms the oral (internal side) and labial (external side) surfaces of the sheath. When the jaw sheaths are closed, the edge of the lower jaw fits against the inner curvature of the upper jaw.

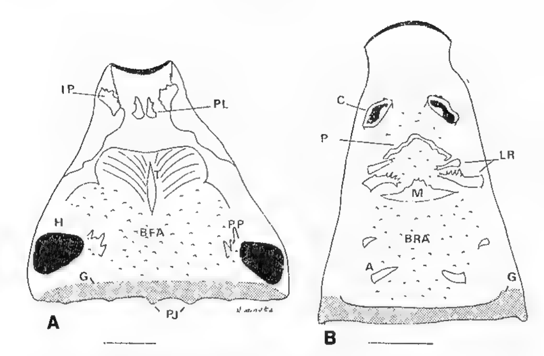


Fig. 2. — Camera lucida drawings of the (A) floor and (B) roof of the oral cavity, stage 37. A: marginal arena papilla; BRA: buccal roof arena; BFA: buccal floor arena; C: choana; G: area of secretory pits; H: buccal pocket; IP: infralabial papillae; LR: lateral ridge; M: median ridge; P: pre-median ridge, PJ: projections; PL: prelingual papillae; PP: prepocket papillae, T. tongue anlage. Scale line: 500 µm.

There is one upper and two lower labial tooth ridges with a modified stratified epithelium. The columns of cells that develop teeth are positioned in the core of this epithelium (fig. 5D). The sequence of cells from the base of a tooth ridge to the top is as follows. basal column cells near the basal membrane, cylindrical odontoid cells with a cornified distal edge, 1-3 cells in different stages of keratinization and pigmentation, and 1-4 labial teeth. Few cells occur in the columns for rows A-1 and P-2 and these may be absent (figs 5C-D) The external and apical surface of the tooth ridge for A-1 is covered by a stratified epithelium (3-4 layers) that continues backwards as a bistratified epithelium. The inner and external faces of the posterior labial ridges are covered by stratified epithelium.

Remains of macrophytes of the family Cyperaceae and pieces of filamentous green algae were found in the intestine (figs 6A-B). Starch grains typical of macrophytes were seen in the histological preparations observed with polarized light, and diatom frustules were also found.

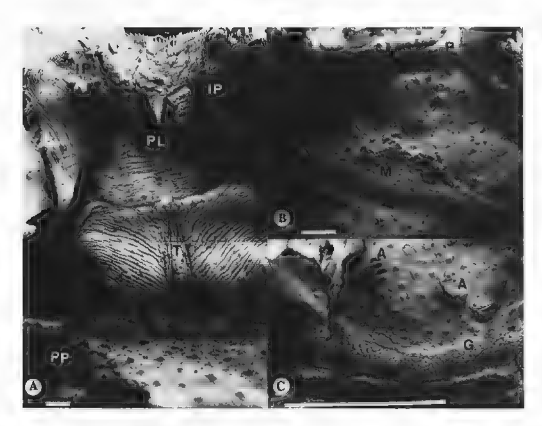


FIG. 3. — (A) SEM micrograph of the floor of the oral cavity, stage 30. Scale line: 100 µm. (B) SEM micrograph of the median ridge and pre-median ridge of the buccal roof, stage 30. Scale line: 100 µm (C) Glandular zone of the posterior part of the buccal roof, stage 30. Scale line: 1000 µm. A: buccal roof arena papilla; G: glandular zone; IP: infralabial papilla; M. median ridge, P: pre-median ridge, PL prelingual papilla; PP pre-pocket papillae; T: tongue anlage.

DISCUSSION

Labial tooth histogenesis in *Hyla minuta* occurs in the same pattern as in other species (e.g., *Bufo arenarum* and *Rana pipiens*), but the final result is different in each row. During the development of *B. arenarum*, histogenesis of the jaw sheaths and labial tooth is continuous (FIORITO DE LOPEZ & ECHEVERRÍA, 1989). The spatial sequence of the cells in each column in the sheaths of *Rana pipiens* represents a chronological sequence by their differentiation from basal epithelial cells (LUCKENBILL, 1965). In *Hyla minuta* the jaw sheath column cells are similar in size to those in *R. pipiens* and *B. arenarum*, but when labial ridge column cells of *Hyla minuta* are compared with those of *B. arenarum*, several differences are evident at the same stages. Labial ridge columns are shorter in *Hyla minuta* and component cells are not produced continuously throughout the larval period. The labial tooth columns of *H minuta* also differ from those of *B. arenarum* by the

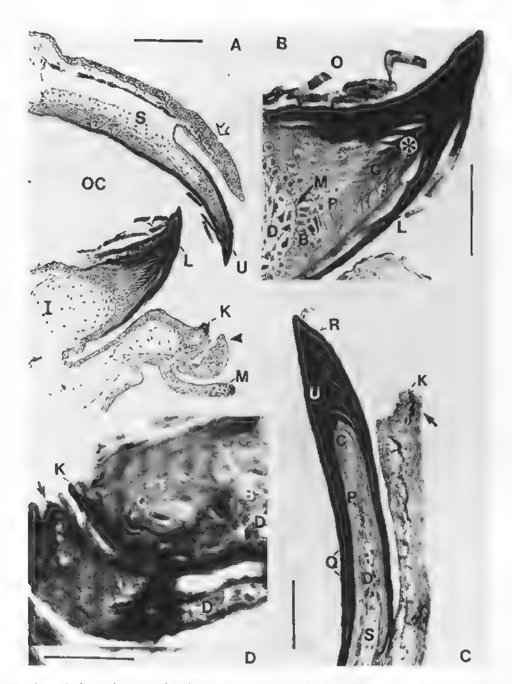


Fig. 4 — (A) Sagittal section of the buccal apparatus, stage 32. Scale line: 100 µm. Closed arrowhead. second lower tooth ridge, open arrow first upper labial ridge, I: infralabial cartilage; K· labial tooth; L· labial surface of lower jaw sheath; M: marginal papilla; OC: buccal cavity; S. suprarostral cartilage, U. upper jaw sheath (B) Sagittal section of lower jaw sheath, stage 32. Scale line: 100 µm. Asterisk: column of tooth cells in process of keratinization; B. basal cells; C cone cells, D dermis; O: oral surface of lower jaw sheath; P: precone cells. (C) Sagittal section of the (U) upper jaw sheath and (arrow) A-1 labial ridge, stage 32. Scale line· 50 µm. C: cone cells; D: dermis; K: labial tooth; P: precone cells; Q: keratinized oral surface of upper jaw sheath, R jaw serration, S: suprarostral cartilage. (D) Transverse section of (K) P-1 with labial teeth and (arrow) P-2 labial ridge with a tooth emerging, stage 32. Scale line: 30 µm. B basal cells; C. keratinization; D· dermis; T: odontoid cell.

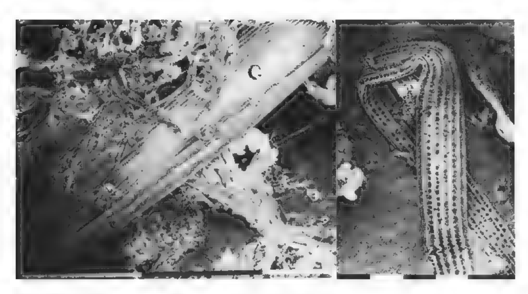


Fig. 5. — SEM micrographs of intestinal contents showing (A) a piece of a cyperaceous plant (C; scale bar: 0 1. mm) and (B) a piece of a filamentous alga (scale bar: 10 µm).

discontinuity of the column, and the morphological cell types forming each column are less evident than in *B. arenarum*. The distance between column cells differs according to the row being examined.

Compared with oral histogenesis of other species (LUCKENBILL 1965; FIORITO DE LOPEZ & ECHEVERRÍA, 1989), the formation of labial teeth in *Hyla minuta* is short-lived, especially in row A-1. Within these differences, the order of appearance of the tooth rows does not seem to fit the general pattern of appearance of the tooth rows that THIBAUDEAU & ALTIG (1988) proposed for 2/3 formulas. In *H. minuta* tadpoles at stage 25, P-1 may be the only row, but labial tooth row formulas of 0/1, 1/1 and 1/2 have been reported for specimens from Iguazú National Park (see also BOKERMANN, 1963; CEI, 1980; HEYER et al., 1990; MONTANELLI, 1991; KAPLAN, 1994), and DUELLMAN (1978) reported 0/2 for a population from Ecuador. It is possible to find more than one labial tooth row formula in certain species at the same stage, but tooth density and distribution usually do not change very much among specimens at the same stage and row (ECHEVERRÍA et al., 1987) *Hyla crucifer* has few teeth in P-3, and up to 50 % of the tadpoles may normally have a formula 2/2 instead of 2/3 (GOSNER & BLACK, 1957). BRESLER (1954) reported that abnormalities of labial teeth and jaw sheaths occurred in tadpoles of *Rana berlandieri* and in *Bufo cognatus* more frequently at higher developmental temperatures; spotty distributions and absences of teeth and jaw sheaths also occur. This is not the case in *H. minuta*, and the situation in this species may represent a polymorphism.

Hylid tadpoles have 0, 2 or 4 lingual papillae (WASSERSUG, 1980; LAVILLA & FABREZI, 1987; HERO, 1990; HEYER et al., 1990; ECHEVERRÍA & MONTANELLI, 1992) Hylid tadpoles that lack lingual papillae (including *Hyla minuta*) include some tadpoles with labial teeth reduced or absent: e.g., *Hyla ebraccata, H. sarayacuensis, H. mixe, H. microps, H.*

microcephala (Wassersug, 1980) and some non-feeding tadpoles (Wassersug & Duellman, 1984). *Hyla minuta* tadpoles share some of the larval features found in *H. sarayacuensis* (*H. leucophyllata* group). few teeth, reduction of oral papillation and roof and floor arena papillae, and absence of lingual papillae. Tooth formation is abbreviated in *H. minuta*, at least for rows A-1 and P-2, and they can be considered vestiges even if the tooth ridges are well developed. Several authors (Heyer & Crombie, 1979; Lannoo et al., 1987; Wassersug, 1980; Heyer et al., 1990) refer to tiny or weakly developed teeth relative to tadpoles with dispersed or few labial teeth.

Conversely, the jaw sheaths of *H. minuta* are strong and well pigmented and keratinized, which suggests that they are efficient cutting instruments for harvesting large pieces of material by biting or ripping pieces from a substrate. The internal structure of the sheaths also supports this idea. Similar species may include *Hyla phlebodes* and *H. sarayacuensis* (Wassersug, 1980). The shape of the borders of the plant pieces found in the larval intestine of *H. minuta* indicates that they were cut with the jaw sheaths.

Conclusions

The labial teeth of *Hyla minuta* are short and weakly pigmented with a short basal sheath, and each tooth head has 3-5 sharp cusps. The tooth ridges have few columns of cells and each column produces few tooth generations. The development of labial teeth is abbreviated at least in some rows (A-1 and P-2).

Labial tooth rows have different tooth densities. Row A-1 has widely spaced teeth that may be distributed all along the tooth ridge. Row P-1 is well developed and most common with teeth distributed throughout the length of the tooth ridge. Teeth present in row P-2 row occur in sporadic patches of 3-5 teeth.

Judging from the anatomical features of the buccal cavity and oral apparatus of these tadpoles and their intestinal contents, I suggest that the tadpole of *Hyla minuta* functions as a herbivorous macrophagous feeder.

Resumen

Las observaciones del aparato bucal y de la cavidad oral de las larvas de *Hyla minuta*, efectuadas con microscopio óptico y electrónico de barrido, han revelado nuevos caracteres anatómicos para tomar en consideración. Las columnas formadoras de dientes (o columnas de células) de las hileras A-1 y P-2 son escasas. Cada columna presenta un número bajo de células epiteliales que darán desarrollo a los dientes córneos. Estos son cortos, sin cuello, y presentan poco pigmento. Cada espátula tiene de tres a cinco dentículos cortos y carenados, ubicados en el extremo distal. En el pico, las columnas formadoras de dientes están bien desarrolladas y muestran una acumulación de células cónicas en diferentes estadios de queratinización que refuerzan sus extremos y las paredes laterales. El pico superior es delgado y su extremo es filoso; el pico inferior es agudo y

presenta una posición proclive cuando se halla inactivo. Los dientes del pico tienen una cúspide. El desarrollo de los dientes labiales parece estar abreviado o incompleto, y no sería continuo durante la ontogenia. Cada hilera de dientes labiales puede presentar diferente densidad dentaria. La distribución de los mismos sobre los pliegues labiales de A-1, P-1 y P-2 puede estar incompleta o ausente en A-1 y en P-2. Las características de la cavidad oral son las siguientes: papilas del arena del techo y del piso de la boca altas y escasas, sin papilas linguales; velo ventral con proyecciones marginales reducidas; puentes lateral y medio presentes; fosetas glandulares en el velo dorsal y en la región posterior del techo bucal. En el contenido intestinal se hallaron algas perifíticas, grandes trozos de algas filamentosas, de cyperaceas (Cyperaceae), y de tejido meristemático, y gránulos de almidón, que permiten considerar a la larva de *H. minuta* con una tendencia trófica del tipo macrófago hervíboro, por lo menos en términos cualitativos. El pico córneo es el elemento del aparato bucal de *H. minuta* que tiene más importancia en la supervivencia de la larva.

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LITERATURE CITED

ALTIG, R., 1970 — A key to the tadpoles of continental United States and Canada. *Herpetologica*, **26**: 108-207.

ALTIG, R & JOHNSTON, G. F., 1989 — Guilds of anuran larvae: relationships among developmental modes, morphologies and habits. *Herp. Mon.*, **3**. 81-109

BEAUMONT, A & DEUNFF, J., 1959 — La kératinisation des dents et du bec larvaires chez *Alytes obstetricans* L. *Arch. Anat. Micr Morph. exp.*, **48**: 307-324

BOKERMANN, W C A., 1963. — Girinos de anfíbios brasileiros. I. (Amphibia-Salientia). *Anais Acad. bras. Cienc.*, **35**: 464-474.

BOURGES, M. & BACHELERIE, C., 1974. Rôle des cellules épithéliales adjacentes aux colonnes dentaires dans la dynamique des odontoïdes cornés chez le têtard d'*Alytes obstetricans* Laurenti. *C. r. Soc. Biol.*, **168**: 1335-1339

BRESLER, J., 1954. — The development of labial teeth of salientian larvae in relation to temperature. *Copeia*, **1954**: 207-211.

CEI, J. M., 1980. — Amphibians of Argentina. *Monit. zool. ital.*, Mon 2 1-609

CHENG, H., 1964. — *The development of the horny beak in* Rana pipiens *tadpoles.* Thesis, Univ. Iowa

DEUNFF, J. & BEAUMONT, A., 1959. — Histogénèse des dents et du bec cornés chez les larves de *Discoglossus pictus* Otth. *C. r. Soc. Biol.*, **153**: 1162-1164.

DUELLMAN, W. E., 1978. — The biology of an equatorial herpetofauna in Amazonian Ecuador. *Misc Publ. Mus. nat. Hist. Univ Kansas*, **65**: 1-352.

Echeverría, D. D., 1992 — Microscopia electrónica de barrido del aparato bucal de la larva de *Hyla pulchella pulchella* (Anura, Hylidae) *Cuad Herp.*, **7**: 24-29.

Echeverría, D. D., Fiorito de Lopez, L E., Vaccaro, O. B & Filipello, A. M., 1987. — Consideraciones acerca de las fórmulas dentarias de las larvas de *Bufo arenarum* Hensel (Anura, Bufonidae). *Cuad. Herp.*, **3**: 33-39.

Echeverría, D. D & Montanelli, S. B., 1992 — Estereomorfología del aparato bucal y cavidad oral de las larvas de *Ololygon fuscovaria* (Lutz, 1925) (Anura, Hylidae). *Rev. Mus. arg Ci nat. Bernardino Rivadavia*, (Zool.), **16**: 3-13.

Fiorito de Lopez, L E & Echeverría, D D , 1984. — Morfogénesis de los dientes larvales y pico córneo de *Bufo arenarum* (Anura, Bufonidae) *Rev Mus arg Ci. nat. Bernardino Rivadavia*, (Zool.), **13**: 573-578

----- 1989. — Microanatomía e histogénesis del aparato bucal en las larvas de *Bufo arenarum* (Anura, Bufonidae). *Cuad. Herp.*, **4**: 4-10.

Gosner, K L., 1960 — A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, **16**: 183-190.

Gosner, K. L. & Black, I. H , 1957. — Larval development in New Jersey Hylidae. *Copeia*, **1957**: 31-36

Hero, J. M., 1990. — An illustrated key to tadpoles occurring in the central Amazon rainforest, Manaus, Amazonas, Brasil. *Amazonia*, **11**: 201-262.

Heyer, W. R. & Crombie, R. I., 1979. — Natural history notes on *Craspedoglossa stejnegeri* and *Thoropa petropolitana* (Amphibia: Salientia, Leptodactylidae) *J Washington Acad. Sci.*, **69**: 17-20

Heyer, W. R., Rand, A. S., Gonçalves da Cruz, C. A., Peixoto, O. L. & Nelson, C. E., 1990. — Frogs of Boracéia. *Arq. Zool.*, **31** (4): 231-410.

Kaplan, M., 1994. — A new species of frog of the genus *Hyla* from the Cordillera Oriental in northern Colombia with comments on the taxonomy of *Hyla minuta*. *J. Herp* , **28**: 79-87.

Kuang, H. C , 1975 — Development of beaks of *Rana pipiens* larvae. *Anat. Rec.*, **182**: 401-414.

Lannoo, M. J., Townsend, D. S. & Wassersug, R. J., 1987. — Larval life in the leaves: arboreal tadpole types, with special attention to the morphology, ecology, and behavior of the oophagous *Osteopilus brunneus* (Hylidae) larva *Fieldiana. Zool.*, (n. s.), **38**: 1-31.

Lavilla, E. O , 1990. — The tadpole of *Hyla nana* (Anura: Hylidae). *J. Herp.*, **24** 207-209.

Lavilla, E. O. & Fabrezi, M., 1987. — Anatomía de las larvas de *Hyla pulchella andina* (Anura: Hylidae). *Physis*, **45B**: 77-82.

Luckenbill, H. C , 1965 — Morphogenesis of the horny jaws of *Rana pipiens* larvae. *Dev. Biol.*, **11**: 25-49.

Martoja, R. & Martoja-Pierson, M , 1957 — *Técnicas de histologia animal.* Barcelona, Torray-Masson: 1-350.

Montanelli, S. B , 1991. — Estudio preliminar de las larvas de *Hyla minuta* Peters, 1872 (Anura, Hylidae) de la selva subtropical argentina. *Bol. Asoc. herp. arg.*, **7**: 6.

Thibaudeau, G. & Altig, R., 1988 — Sequence of ontogenetic development and atrophy of the apparatus of six anuran tadpoles. *J. Morph.*, **197**: 63-69.

Viertel, B., 1982. — Oral cavities of central European anuran larvae (Amphibia): morphology, ontogenesis and generic diagnosis. *Amphibia-Reptilia*, **4**: 327-360.

Wassersug, R. J., 1980. — Internal oral features of larvae from eight families: functional, systematic, evolutionary and ecological considerations *Misc. Publ. Mus. nat. Hist. Univ. Kansas*, **68**. 1-146.

Wassersug, R. J. & Duellman, W. E., 1984. Oral structures and their development in egg-brooding hylid frog embryos and larvae: evolutionary and ecological implications. *J. Morph.*, **182**: 1-37.

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Sexual size and shape difference in the crested newt (*Triturus carnifex*): ontogenetic growth aspects

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The patterns of emergence and development of sexual size and shape differences (SSSD) in the crested newt were examined. The results of our study indicate that differences in size and shape between females and males appear mostly in the period between the first and the second hibernation, which seems particularly important for development of SSSD. Concerning the questions of time and speed of morphological divergence between sexes, we can conclude that the establishment of SSSD precedes reproduction. The process is not gradual; two periods can be distinguished. The second period is characterized by rapid change, resulting in considerable intersexual differences in various parameters. Juveniles exhibited pronounced sexual dimorphism of growth rates, as well as differences in level and timing of departures from isometric growth, and in values of the Wolterstorff index (WI). The existence of two distinct periods in development of SSSD was also confirmed at the multivariate level.

Introduction

European newts (*Triturus*, Salamandridae) have the most pronounced morphological sexual dimorphism among tailed amphibians, especially during the breeding season. Sexes differ in coloration, skin texture and glandular development, as well as in body size and shape. In most of 12 extant species, including *Triturus carnifex*, females are larger than males (Kalezić et al., 1992). Significant differences were found for most linear dimensions (measures of size), as well as for some ratios of these dimensions (measures of shape), though shape differences have attracted less interest so far. It was established that the extent of sexual size difference in European newts is a variable condition not constrained by species body size, spatial proximity of analysed populations or altitude (Kalezić et al., 1992).

However, patterns of emergence and development of sexual size and shape differences (SSSD) still remain to be clarified. Many questions can be raised concerning the time and speed of morphological divergence between sexes. Do the differences in body size and shape fully develop prior to attainment of sexual maturity? Is the process of divergence

gradual during the juvenile phase of life or can a distinct period of rapid change be observed? Moreover, it is still unknown which morphometric characters are most involved in the above process.

Growth before the age of first reproduction (larval and juvenile stages) appears to be the major determinant of body size in amphibians and reptiles in general (Halliday & Verrell, 1988), and in newts in particular (e.g. Hagstrom, 1980; Glandt, 1981; Verrell & Francillon, 1986; Kalezić et al., 1994).

Thus, as the main concern of this paper was to find the pattern of SSSD appearance in *Triturus carnifex*, we have studied morphometric growth aspects during the critical ontogenetic period between the first and the second hibernation.

Materials and methods

Laboratory procedures

Crested newt specimens were collected from Lokanj pond (Montenegro) in October 1991. We sampled 43 juveniles, as well as fully mature individuals, 21 females and 19 males ("adults" in the following text). Newts were anaesthetized by immersion in a 2:1000 MS-222 (Sandoz) solution, individually marked by toe clipping and measured. Shortly after measuring, the newts were put into hibernation in a cold room at 7.5°C till next February. On emergence from "wintering", 43 juveniles (all survived hibernation) were measured again and housed in a 300 l aquarium. This aquarium had been established three years before and contained diverse weeds, simulating a natural pond. It was exposed to a natural photoperiod and daily changes in room temperature (10-25°C); the constant water level was maintained. Water was continually recycled and filtered. Thus, the newts were maintained in standard conditions with ad libitum access to food (earthworms and pieces of beef meat). Juveniles were measured each subsequent 30 days for another 10 months. Body mass was determined by blotting individuals dry and weighing them to the nearest 0.01 g

Out of 43 juveniles, 23 survived till the second hibernation, 15 females and 8 males. They were sexed according to the presence of secondary sexual characteristics, the identification of males being easier through the appearance of a dorsal crest, a thin crenulated skin flap, a bluish-white streak along the tail and a swollen black cloaca. At that age, exclusively female secondary sexual feature, the presence of cloaca papillae, was less apparent.

Morphometric variables

In juveniles and adults, 9 morphometric characters were measured: SVL (snout-vent length), Lcp (the distance from the snout to the frontal edge of the cloaca), Ltc (head width at the angle of the jaw), Lc (head length from the snout to the corner of mouth),

Lc1 (distance between the snout and the gular skin fold), Pa (fore limb length, measured from axilla to the tip of the longest finger), Pp (hind limb length, from groin to the tip of the longest finger), D (distance between fore and hind limbs) and Lh (the maximum fin height measured at the base of the tail). All measurements were made with a dial caliper with 0.1 mm precision.

STATISTICAL ANALYSIS

Two data sets are presented here: one consists of records of the same individual at different time points, the other is based on samples of different individuals at the same time point. According to COCK (1966), they are defined as longitudinal and static data, respectively. Consequently, we distinguish growth allometry, changes in size-shape relationships with time, from static allometry, based on records of different individuals (e.g. GOULD, 1966).

The growth of juvenile newts was expressed through relative growth rate (K), calculated according to the following equation (ANDREWS, 1982).

$$K = (\ln S_2 - \ln S_1) / (t_2 - t_1),$$

where S_1, S_2 and t_1, t_2 were size (standard length) at and time of two measure points. Linear regressions of SVL on time were also calculated for both groups of juveniles

The Wolterstorff index (WI), widely recommended as a useful tool for distinguishing crested newt taxa (e.g. HERRE, 1932; SOVA, 1973, KALEZIĆ et al., 1990; LANZA et al., 1991), was calculated as the ratio of forelimb length to interlimb distance (WOLTERSTORFF, 1923). Apart from various uni- and bivariate statistical analyses (SOKAL & ROHLF, 1981; ZAR, 1984), multivariate procedures of principal-component analysis (PCA) and Mahalanobis distance (D^2) were applied. Due to the small sample size, multivariate procedures were conducted on the set of seven morphometric traits (SVL, Ltc, Lc1, Pa, Pp, D, Lh) in juveniles and adults.

Principal-component analysis allowed simultaneous analysis of morphometric data, reducing dimensionality but retaining variation. It was performed on the variance-covariance matrix of log-transformed data, a procedure recommended when dealing with morphometric traits (e g. BOOKSTEIN et al., 1985). Separate principal-component analyses were computed for 4 time points (I: prior to the first hibernation; IV: April; VIII: August; XI: November, prior to the second hibernation) in juveniles and for adults. The Mahalanobis multivariate distance between the sexes was computed for all measurement points. Mantel test was used to analyse the correspondence of character variance-covariance matrices.

RESULTS

The range of standard length of 43 juveniles was 44.1-55.9 mm (mean ± standard error: 51.6 ± 0.4 mm). This indicates that they hatched in the same season (spring 1991),

metamorphosed during a relatively short time span and therefore were suitable for this study, though there is evidence of a plastic life-history including facultative paedomorphosis in the Lokanj population (Kalezić et al., 1994). The mean SVL of 23 surviving juveniles at the begining of the study was 51 7 ± 0.6 mm (range 47.9-55.9 mm) for females and 51.1 ± 0.6 mm (range 48.8-53.7 mm) for males, the difference being statistically insignificant (ANOVA, F – 0.445, $P > 0.05$). The mean value of SVL prior to the second hibernation was 75.2 ± 0.8 mm (range 69.7-79.1 mm) and 69.9 ± 1.9 mm (range 56 9-73.8 mm) for females and males, respectively. Intersexual difference was significant (ANOVA, $F = 8.74$, $P < 0.01$)

It was essential for our laboratory study to avoid competition for food among juveniles and therefore allow the undisturbed development of SSSD. When density is low (intraspecific competition low or absent), a normal distribution of individual growth rates is expected. Otherwise, intense competition for food would result in a skewed or lognormal distribution (Wilbur & Collins, 1973; Wilbur, 1976). The distribution of growth rates in our data set showed good fit to normal distribution (Kolmogorov-Smirnov test, $P >$ 0.05 in all cases). Thus, we can conclude that nutritional conditions were similar for all individuals, i.e. there was no size-dependent advantage.

The analysis of relative growth rates indicated the existence of two distinct periods. Up to July (the first half of the year) females invariably had faster growth, but the difference was insignificant (ANOVA, $P >$ 0 05 in all cases). The second period is characterized by significantly increasing differences (ANOVA; July-August, P – 0.01; August-September, $P = 0.05$; September-October, $P < 0.01$), except for the last month before the second hibernation ($P > 0.05$). The comparison of linear regressions of SVL on time over the whole time span studied indicated that females were growing significantly faster than males (b♀ = 2.436 ± 0 100, b♂ = 1.925 ± 0.097; t test, $t = 3.66$, $P < 0.001$).

It is of interest to note negative growth during hibernation. Indeed, the comparison of the first two measurement points showed a significant decrease, not only in weight (paired t test, $t = 15.87$, $P < 0.0001$), but in length as well (paired t test, $t = 4.39$, $P <$ 0.001), with pronounced individual variability.

In the sample of adults, females were larger than males (mean SVL 74.4 ± 1.4 and 72.6 ± 1.0, respectively); however, the difference was insignificant (ANOVA, $F = 1.16$, $P > 0.05$).

Bivariate allometry

In order to analyse the pattern of changes in bivariate allometric coefficients during ontogeny, regression analysis was performed on eight morphometric traits (using SVL as the independent variable) for all measurement points in juveniles, as well as for adults (tab. 1). Intersexual difference in departures from isometric growth is obvious in the number of statistically significant allometric coefficients. In juvenile males, out of 88 values, 18 were statistically significant (20.4 %) vs. 6/88 (6.8 %) in juvenile females (χ^2, P < 0 05). Also, in males, contrary to females, most of the significant values appear in the second half of the year. Negative allometry is apparent: 5/6 significant coefficients in

Tab. 1. - Significant bivariate allometric coefficients in males and females. Positive allometry: +, $P < 0.05$; ++, $P < 0.01$. Negative allometry: –, $P < 0.05$; – –, $P < 0.01$; – – –, $P < 0.001$. I - XI: measurement points in juveniles; AD: adults.

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	AD
♂												
Lcp				–					–	– – –		
Ltc		+					– –		– –	– –	– –	
Lc										–		
Lc1				–						–	– –	
Pa			–			+			++			
Pp												
D							+	+				
Lh							+					+
♀												
Lcp				++								
Ltc												
Lc		–										
Lc1												–
Pa				–							–	
Pp	–										–	– – –
D												
Lh												

juvenile females, 2/2 in adult females and 12/18 in juvenile males. Traits showing considerable ontogenetic allometry are: Lcp, Ltc, Lc1 and Pa in males, Pa and Pp in females.

WOLTERSTORFF INDEX AND MAHALANOBIS DISTANCE

Morphometric differentiation between the sexes in terms of multivariate distance (Mahalanobis D^2) and bivariate parameter WI (Wolterstorff index) is shown in fig. 1. Mahalanobis distance changed slightly between measurements I and VII (prior to the first hibernation and next July) and then increased rapidly. At the time of the second hibernation it was even higher than the value found in the sample of adults ($D^2_{ad} = 6.02$).

Intersexual differences in WI values, characteristic for some salamander species (particularly crested newts), were also calculated for all measurement points. The pattern of change followed that of Mahalanobis distance – rapid change in the second half of the year resulted in highly significant differences between sexes. The value just prior to the second hibernation (0.071) was somewhat higher than in adults (0.062).

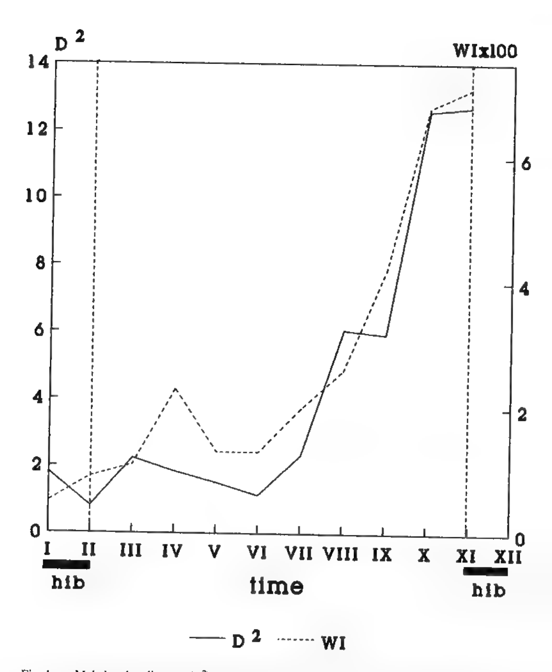


Fig 1. – Mahalanobis distance (D^2) and differences in values of Wolterstorff index (WI) between juvenile males and females, hib: hibernation. Measurement points II – XI correspond to months (February to November).

DEVELOPMENT OF SSSD IN MULTIVARIATE SPACE

Mantel test was applied to examine the correspondence of character variance-covariance matrices between the sexes. The absence of significant positive correlations indicated the differences in character covariance structure between females and males, juveniles as well as adults.

The results of PCA showed that the first two principal components account for a considerable amount of total variation: 74.2-95.1 % in juvenile males, 91.0 % in adult males, 78.7-86.2 % in juvenile females and 88.7 % in adult females. This indicates strong correlations between variables, as is expected when dealing with morphometric traits. Principal-component 1 can be considered a general size component, while PC2 represents a shape component. The magnitudes of eigenvector elements indicate the contribution of each original trait to principal components (fig. 2).

Principal-component 1 measures variation attributable to differences in size. All traits load on PC1 positively. Similar, but not identical, values of eigenvector coefficients indicate the influence of allometric information. The most striking feature is the position of fin height (Lh) in PC1-PC2 space. This trait dominates both axes and is opposed to all other traits (this is less pronounced only for measure point IV in females). It has a constant, stable position in morphological space, irrespective of sex, the only exception being adult males. In adult males, the position of sets of traits is reverse, compared to females, due to opposite signs of eigenvector loadings on PC2.

Traits related to locomotion, Pa and Pp, load as a group and show some intersexual differences. Other traits do not show a recognizable pattern. Snout-vent length has a relatively stable position in juveniles, irrespective of sex. Variation in head dimensions, related to feeding (Ltc and Lc1) and in interlimb distance (D) shows no simple trend during ontogeny, though intersexual differences appear in the youngest juveniles (measurement points I and IV).

The average values of PC scores (fig. 3) show time-related changes in size and shape of studied individuals. The last pair of points represents adult males and females occupying different morphological spaces; shape divergence is mostly influenced by one trait, fin height.

THE ATTAINMENT OF SEXUAL MATURITY

The newts with well expressed secondary sexual characteristics were allowed to court and oviposit shortly after the second hibernation. We observed courtship and spermatophore deposition, but no oviposition took place during the expected breeding season.

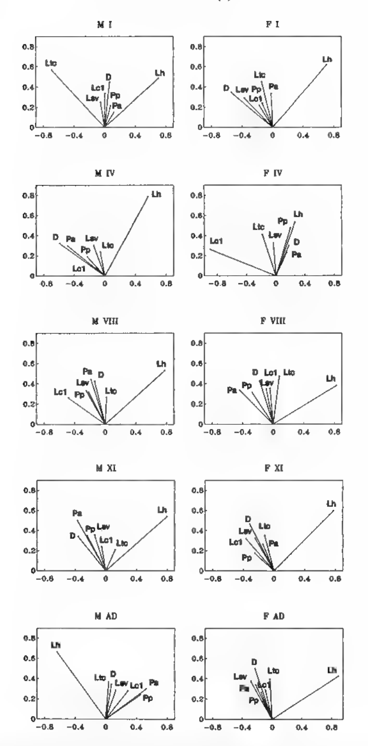


Fig. 2. - Plots of eigenvectors (X axis PC2, Y axis: PC1) for 4 measurement points in juveniles (I, IV, VIII, XI) and for adults (AD); M: males, F: females.

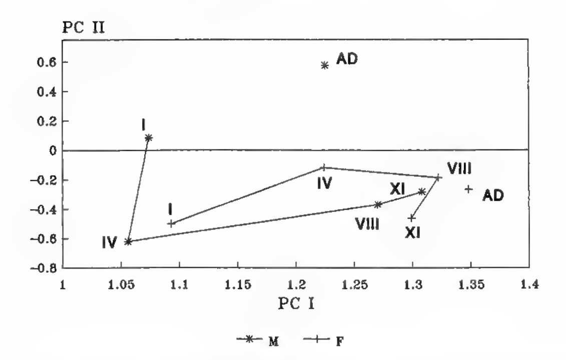


Fig. 3. Average values of PC scores for 4 measurement points in juveniles (I, IV, VIII, XI) and for adults (AD); M: males, F: females.

DISCUSSION

The results of our study indicate that differences in size and shape between females and males appear mostly in the period between the first and the second hibernation, which seems particularly important for development of SSSD. At least some juvenile crested newts, maintained in our laboratory, attained sexual maturity at the age of 2 years. This seems to be the minimum age at first breeding for *Triturus cristatus* superspecies; in the field it usually takes longer – from 3 to 5 years (HAGSTROM, 1975; FRAZER, 1983; FRANCILLON-VIEILLOT et al., 1990).

Thus, concerning the questions of time and speed of intersexual morphological divergence, we can conclude that the establishment of SSSD precedes reproduction The process is not gradual; a period of rapid change can be distinguished, resulting in considerable intersexual differences, confirmed by various uni-, bi- and multivariate analyses.

In species with intersexual size difference, several patterns of juvenile growth are possible (ANDREWS, 1982). One is for juveniles to grow at similar rates until individuals of the smaller sex reach asymptotic length, while the larger sex continues to grow, so the growth curves diverge. Another pattern is to grow at different rates, either from the beginning (hatching) or from some point early in life. In our study, juveniles exhibited a

pronounced sexual difference in growth rates, according to the second pattern. Greater importance of juvenile compared to adult growth rates has been stressed before (e.g. HALLIDAY & VERRELL, 1988).

As far as adults are concerned, in the crested newt populations females are almost invariably larger than males (KALEZIĆ et al., 1992). In this sample of Lokanj adults, the difference is not significant However, another sample from the same population obtained significant sexual size difference (unpublished results). The variation of body size distributions and the extent of sexual size dimorphism at the inter- and intrapopulation level is a common phenomenon (HALLIDAY & VERRELL, 1988; STAMPS, 1993). Many factors can be responsible for this variation among the samples from the same population, such as differences in growth rates or age structure (STAMPS, 1993).

Our results show a substantial sexual size divergence during the critical period prior to sexual maturation, but the interpretation of adult size data requires more detailed information on adult growth patterns.

Intersexual shape differences were revealed by various bi- and multivariate methods. In terms of bivariate allometry, considerable differences in level and timing of departures from isometric growth are found between females and males. Allometry is mostly negative with respect to standard length. However, allometric coefficients for Pa, the trait involved in WI, show intersexual differences. If we compare all coefficients, values are almost without exception negative in females and positive in males. Hind limb length (Pp) shows mostly isometric (to slightly negative allometric) growth in males and negative allometric growth in females. This confirms some previous findings. REHAK (1983) found for the crested newt females relative shortening of legs with respect to body length. This difference in limb lengths between the sexes might be associated with the courtship behaviour in males and sperm transfer (e.g. HALLIDAY, 1977; REHAK, 1983; RAXWORTHY, 1989).

The existence of two distinct periods in development of SSSD was confirmed at the multivariate level (Mahalanobis distance). Differences in values of the Wolterstorff index exhibited the same pattern, which confirms that this bivariate parameter is a good indicator of size-shape changes. Principal-component analysis showed that the trait with the largest contribution to both components, PC1 and PC2, was fin height (Lh). Sets of traits had a reverse position in adult females and males, occupying different morphological spaces, mainly due to shape differences

We are aware that the paucity of sample size in our study, especially of males, precludes well-supported conclusions, though general trends are apparent. Additional data are needed for a more rigorous assessment of these trends.

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LITERATURE CITED

ANDREWS, R. M., 1982. – Patterns of growth in reptiles. *In*: C. GANS & F. H. POUGH (ed.), *Biology of reptiles*, New York, Academic Press, vol. **13**: 273-320.

BOOKSTEIN, F., CHERNOFF, B., ELDER, R., HUMPHRIES, J., SMITH, G. & STRAUSS, R., 1985. – *Morphometrics in evolutionary biology*. Philadelphia, Pennsylvania, Academy of Natural Sciences of Philadelphia, Special Publication **15**.

COCK, A. G., 1966. – Genetical aspects of metrical growth and form in animals. *Q. Rev. Biol.*, **41**: 131-190.

FRANCILLON-VIEILLOT, H., ARNTZEN, J. W. & GÉRAUDIE, J., 1990. – Age, growth and longevity of sympatric *Triturus cristatus*, *T. marmoratus* and their hybrids (Amphibia, Urodela): a skeletochronological comparison. *J. Herp.*, **24**: 13-22.

FRAZER, D., 1983. – *Reptiles and amphibians in Britain*. London, Collins: 1-256.

GLANDT, D., 1981. – Zum Postmetamorphose-Wachstum von *Triturus cristatus* (Amphibia, Urodela, Salamandridae) im Freiland. *Zool. Jb. Anat.*, **106**: 76-86.

GOULD, S. J., 1966. – Allometry and size in ontogeny and phylogeny. *Biol. Rev.*, **41**: 587-640.

HAGSTROM, T., 1975. – Notes on growth and age distribution of adult *Triturus vulgaris* L. and *T. cristatus* Laurenti (Urodela, Salamandridae) in SW Sweden. *Bull. Soc. zool. Fr.*, **100**: 680.

----- 1980. – Growth of newts (*Triturus cristatus* and *Triturus vulgaris*) at various ages. *Salamandra*, **16**: 248-251.

HALLIDAY, T. R., 1977. – The courtship of European newts: an evolutionary perspective. *In*: D. H. TAYLOR & S. I GUTTMAN (ed.), *The reproductive biology of amphibians*, New York, Plenum Press: 185-232.

HALLIDAY, T. R. & VERRELL P. A., 1988. – Body size and age in amphibians and reptiles. *J. Herp.*, **22**: 253-265.

HERRE, W., 1932. – Vergleichende Untersuchungen an den Unterarten des *Triturus cristatus* Laur. *Z. Anat. Entw.-Gesch.*, (1), **99**: 1-62.

KALEZIĆ, M. L., CRNOBRNJA, J., ĐOROVIĆ, A. & DŽUKIĆ, G., 1992. – Sexual size difference in *Triturus* newts: geographical variation in Yugoslav populations. *Alytes*, **10**: 63-80.

KALEZIĆ, M. L., CVETKOVIĆ, D., ĐOROVIĆ, A. & DŽUKIĆ, G., 1994. – Paedomorphosis and differences in life-history traits of two neighbouring crested newt (*Triturus carnifex*) populations. *Herp. J.*, **4**: 151-159.

KALEZIĆ, M. L., DŽUKIĆ, G., STAMENKOVIĆ, S. & CRNOBRNJA J., 1990. -- Morphometrics of the crested newt (*Triturus cristatus* complex) from Yugoslavia: relevance for taxonomy. *Arh. biol. Nauka*, Beograd, **42**: 17-37.

LANZA, B., GENTILE, E. & TORRICELLI, I., 1991. – Preliminary data on the number of presacral vertebrae in *Triturus cristatus*-Artenkreis (Amphibia: Caudata: Salamandridae). *In*: G. GHIARI et al. (ed.), *Symposium on the evolution of terrestrial vertebrates*, Modena, Mucchi, Selected Symposia and Monographs U.Z.I. **4**: 531-534.

RAXWORTHY, C. J., 1989. – Courtship, fighting and sexual dimorphism of the banded newt, *Triturus vittatus ophryticus*. *Ethology*, **81**: 148-170.

REHAK, I., 1983. – Changes in body measures during the growth of the newts *Triturus vulgaris*, *T. alpestris* and *T. cristatus* (Amphibia: Urodela). *Vest. čs. Společ. zool.*, **47**: 51-67.

SOKAL, R. R. & ROHLF, F., 1981. – *Biometry*. San Francisco, Freeman & Co.: 1-776.

SOVA, C., 1973. – Morphometric researches in the genus *Triturus* from Rumania (Sereth River Basin). *Studii si Comunicari, Muzeul De Stiintele Naturii BacČau*, **1973**: 85-286.

STAMPS, J. A., 1993. – Sexual size dimorphism in species with asymptotic growth after maturity. *Biol. J. linn. Soc.*, **50**: 123-145.

VERRELL, P. A. & FRANCILLON H., 1986. – Body size, age and reproduction in the smooth newt, *Triturus vulgaris*. *J. Zool.*, (A), **210**: 89-100.

WILBUR, H. M., 1976. – Density-dependent aspects of metamorphosis in *Ambystoma* and *Rana sylvatica*. *Ecology*, **57**: 1289-1296.

WILBUR, H. M. & COLLINS J. P., 1973. — Ecological aspects of amphibian metamorphosis. *Science*, **182**: 1305-1314.

WOLTERSTORFF, W., 1923. — Übersicht der Unterarten und Formen des *Triton cristatus* Laur. *Blätter Aquar. Terrarienk. Kde. Stuttgart*, **34**: 120-126.

ZAR, J. H., 1984. — *Biostatistical analysis*. New Jersey, Prentice-Hall: 1-718.

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BOURRET, R., 1942. – *Les Batraciens de l'Indochine*. Hanoï, Institut Océanographique de l'Indochine: i-x + 1-547, pl. I-IV.

GRAF, J.-D. & POLLS PELAZ, M., 1989. – Evolutionary genetics of the *Rana esculenta* complex. *In*: R. M. DAWLEY & J. P. BOGART (eds.), *Evolution and ecology of unisexual vertebrates*, Albany, The New York State Museum: 289-302.

INGER, R. F., VORIS, H. K. & VORIS, H. H., 1974. – Genetic variation and population ecology of some Southeast Asian frogs of the genera *Bufo* and *Rana*. *Biochem. Genet.*, **12**: 121-145.

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